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THE BIOLOGICAL VARIATION OF THE INFECTIOUS LARYNGOTRACHEITIS VIRUS*

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INFECTIOUS laryngotracheitis (IL) was first reported by May and Tittsler[11] in 1925. The disease was observed in a flock of chickens in Rhode Island in October 1923. Following this initial account, the presence of the disease in other areas was reported by Hinshaw[9] in California, Gwatkin[8] in Canada, Seddon[14] in Australia and Van Heelsbergen[15] in Holland. In the first descriptions of the disease, some workers referred to it as infectious bronchitis and thus the early literature is confusing. Since these primary investigations from 1930 to 1940, there has been very little research done on the basic characteristics of the disease and its etiological agent.

Infectious laryngotracheitis is still a problem in many areas. Outbreaks occur in vaccinated as well as in non-vaccinated flocks. The occurrence of the disease in vaccinated flocks raises the question as to whether it is due to variant types of virus, misdiagnoses, deficiencies in the vaccine or failure to properly administer the vaccine.

The etiological agent of IL is a virus which was first isolated by Beaudette[2] and by Beach[1] in 1930. It can be propagated on the chorio-allantoic membrane of an embryonated chicken egg, following a technique described by Brandly.[3]

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The virus produces small gray plaques on the membrane in about 3 days. These areas of thickening become larger up to the 5th to 7th day when the embryo usually dies. There is an accompanying edema of the membrane and the allantoic fluid becomes thickened. Intranuclear inclusions can be found in some of the ectodermal cells of the membrane between the 1st and 3rd days following inoculation.[3,4]

Investigations into the possibility of variant types of IL virus have not received much attention. Pulsford[12] of Australia has reported on the possible P.Q. type variation of the IL virus. Recently, several viruses have been isolated which produce embryo mortality with lesions similar to those reported for IL virus. The virus isolated by Fahey and Crawley[7] is an example. Cover and Benton[5] have reported the isolation of a IL virus (virus 146) which did not show the usual virulence when administered to susceptible birds. Since this time, additional studies have been made with virus 146 and additional similar viruses of low virulence have been isolated. In each case, the isolation was made from birds showing respiratory distress, but neither the individual bird nor the clinical disease syndrome of the flock conformed to the classic description of the acute disease. Birds from some of these flocks when received by the diagnostic laboratory showed signs and lesions similar to chronic respiratory disease. It is the purpose of this paper to describe the less acute form of IL and to give the results of several experiments regarding the biological properties of these viruses.

MATERIALS AND METHODS

In order to confirm the identity of the 146 virus as that of IL virus, its ability to produce the typical inclusion body in the epithelial cells of the trachea was tested. To do this, 0.5 ml of chorio-allantoic fluid was administered intratracheally to each of 15 birds. Three of these birds were killed on the 3rd, 4th, 5th and 6th days following inoculation and specimens of the trachea were taken for examination for inclusion bodies. Such tissues were fixed and embedded and later stained with hematoxylin-eosin in the autotechnicon.

Additional field cases of the less acute type of IL were studied. In such outbreaks, the case history and necropsy findings were similar to those found for the 146 virus. The morbidity and mortality was low with the total mortality including that due to IL for the flock over the broiler growing period of 9 to 10 weeks being less than 8 percent.

The principal signs were those of respiratory distress with little or no gasping. There was no history of blood being expelled from the mouth or nose by coughing. At necropsy, the tracheas were inflamed but did not contain blood. In some birds there was a thin yellow exudate in the trachea which in some cases appeared to be forming into a pseudo-membrane. No tracheal or laryngeal plugs were found. The laryngotracheitis virus was isolated from such cases. The technique for such virus isolations involved making a deep scraping of the tracheal mucous membrane by the use of a small sterile spatula. The material so collected was placed in a tube containing approximately 5.0 ml of nutrient broth and was shaken to break up the large particles. This inoculum was injected into the chorio-allantoic sac of 9 to 11 day embryonated chicken eggs. Prior to its use, the material was stored at –15 C. The material was treated with 10,000 units of penicillin and 10 mg of streptomycin per ml before embryo inoculation. Although a number of isolations were made in this manner, only two were used in the experiments described below. These are viruses 742 and 1040. Virus 742 was isolated from birds showing the typical acute form of IL and it has produced severe clinical response when given to susceptible birds. Virus 1040 was isolated from a less acute case of IL and has not shown the acute pathogenecity of virus 742 and the commercial vaccine strains.

Cross immunity tests were conducted on live birds and by the serum neutralization test in embryonated chicken eggs. Commercial vaccine IL virus A, commercial vaccine IL virus B, virus 146 and virus 1040 C were administered intratracheally in 0.5 ml amounts to each of three birds. On the 14th day post-inoculation three birds in each lot were bled and the serum stored at –15 C for serum neutralization

tests. These tests were made in the usual manner by inoculating 0.1 ml amounts into the chorio-allantoic sac.[6] The embryonated eggs were candled daily and the test was terminated on the 8th day post-inoculation. Titres were determined according to the method of Reed and Muench.[13]

From the same lot of birds, cross immunity tests of live birds were made by challenge with the other viruses. This challenge was made by the injection of 0.1 ml of virus into the infra-orbital sinus. The reactions were read on the 3rd and 4th days post-inoculation. A positive response consisted of a swelling of the sinus and a nasal discharge.[10]

Also from the same lot of birds, tracheal tissue specimens were prepared for the detection of inclusion bodies. One additional virus was included in this test (virus 742). Birds were sacrificed on the 3rd, 4th, 5th and 6th days post-inoculation and tracheal specimens were placed in each of the following fixatives: Zenkers—acetic acid, pH 2.5, Helly's fluid with formalin, pH 3.6, Helly's fluid with neutral formalin, pH 3.8, Bouins fluid, pH 1.4, alcohol 70 percent, pH 9.2 formalin 10 percent, pH 7.0. Tissues were fixed in the above solutions for 24 hours and then transferred to the autotechnicon for processing. They were embedded in tissue mat, cut at 7 microns and the sections were stained in the autotechnicon with hematoxylin eosin.

Two additional tests were made on the 146 virus. A titration of the virus was made of the various portions of the embryonated chicken egg viz, embryo, yolk, chorio-allantoic membrane and chorio-allantoic sac. The dilutions were made in nutrient broth and 9 to 11 day embryonated embryos were inoculated with 0.1 ml into the chorio-allantoic sac. The second experiment was a thermo-stability test from 1 to 5 hours at 37½ C and 25 C respectively. The virus was then injected into the chorio-allantoic sac of 9 to 11 day embryonated chicken eggs to determine its viability.

RESULTS

The typical intranuclear inclusion bodies of IL were

found in the tracheal epithelial cells from birds which had received the 146 virus. These inclusions were most numerous on the 3rd and 4th days post-inoculation and appeared as large, globular, acidophilic bodies. The cellular changes in the tracheal mucosa following the administration of the virus will be described below.

Examination of tracheal tissue from the birds receiving the following viruses: commercial vaccine strain A, commercial vaccine strain B, virus 742, virus 1040, and virus 146, indicated that all of these viruses produced inclusion bodies. The time of the appearance of the inclusion body in relation to the day after inoculation was variable and appeared to depend upon the rate of tissue response of the individual bird rather than upon the virus. There was a difference in the time of tissue reaction for each bird and varied as much as 48 hours in some cases. Thus the exact time post-inoculation that inclusion bodies were found could not be definitely established. The presence of the intranuclear inclusions is a part of the cellular response to IL virus and they appear only in the early stages of the tissue reaction.

The chronological order of the tissue response as seen in this experiment is herewith reported. The first and very dramatic changes were (1) the loss of the cilia from the epithelial cells which are in a pseudostratified layer and (2) the disappearance of the mucous glands. These changes occurred rapidly and produced a striking alteration in the appearance of the tracheal mucosa. The epithelial cells increased in size and shape following these initial changes. At this time the intranuclear inclusion bodies were present. There was an accompanying active hyperemia in the tunica propria; the capillaries were distended with blood. The epithelial layer changed its characteristics from that of a pseudostratified to a stratified type. The individual cells were no longer columnar in shape but polyhedral. As the reaction progressed there was a proliferation of the connective tissue in the tunica propria with an invasion of macrophages and lymphocytes. At this stage the inclusion bodies were almost completely gone. There now was produced an accumulation

of cellular and tissue debris on the tracheal surface (lumen) and a pseudomembrane was formed in the more acute type of reaction. However, the tissue reaction to the 146 virus began to subside before a definite pseudomembrane was formed. In the case of the commercial viruses used in the experiment both showed a more prolonged reaction than that following the 146 virus. There was evidence of hemorrhage into the connective tissue of the tunica propria which, in some cases, was of sufficient size to reach the tracheal lumen. If sufficient hemorrhage occurred, blood could be found on the surface of the trachea. The reaction described above was followed by repair.

All of the fixatives used in this experiment allowed the identification of inclusion bodies. However the fixatives which had a pH in the low range (acid) allowed these bodies to be more clearly and distinctly identified. Those tissues processed through alcohol or formalin did not show the inclusions very clearly and sometimes not at all.

The results of the cross-immunity tests in live birds 19 days after intratracheal exposure indicates that these viruses were similar antigenically. The susceptible control birds showed a definite positive response to the intrasinous challenge while the birds previously exposed to all of the various IL viruses did not. The results of the neutralization tests on serum from birds receiving the different viruses 14 days previously and being tested against each of the three viruses used in the experiment showed that there was little difference in their ability to neutralize the homologous or heterologous viruses. The results are shown in table I.

The thermostability test of the 146 virus indicated that it was not affected by incubation at 25 C or 37.5 C for as much as five hours, since there was no reduction in the ability of the virus to produce plaques and mortality in 9 to 11 day embryonated chicken eggs.

Titration of the embryo, yolk, chorio-allantoic fluid and chorio-allantoic membrane in the chorio-allantoic sac indicated that the 146 virus was most prevalent in the chorio-allantoic membrane and least prevalent in the embryo. The virus titers are given in table II.

TABLE I

Results of serum neutralization tests to check cross immunization between strains of infectious laryngotracheitis virus

Virus used to Vaccinate Birds	Serum Neutralization Index for Virus			
	Virus 146	Commercial A	Commercial B	Virus 1040
146 Virus	1.30	2.25	1.67	1.00
146 Virus	0.24	1.25	1.00	0.00
146 Virus	1.24	3.00	1.18	0.82
Commercial A	1.75	0.75	1.83	1.49
Commercial A	2.25	1.43	0.83	2.00
Commercial B	3.00	2.57	2.87	3.17
Commercial B	2.25	2.67	2.87	No serum
Commercial B	1.20	2.33	1.83	1.49
1040 Virus	2.58	2.42	1.50	1.32
1040 Virus	1.20	2.25	1.50	1.32
1040 Virus	0.67	0.75	1.83	1.32

TABLE II

Titration of 146 virus in various portions of the embryonated chicken eggs following inoculation of the chorio-allantoic sac

	Titer—E.I.D. 50
Test 1	
Chorio-allantoic fluid	3.50
Chorio-allantoic membrane	5.00
Embryo	1.50
Test 2	
Chorio-allantoic membrane	3.50
Yolk	1.83
Embryo	1.50

DISCUSSION

Since the formation of the typical intranuclear inclusion bodies have been found in the tracheal epithelium, the identity of the 146 virus as infectious laryngotracheitis is justified.

Although the data presented here in regard to the antigenic polyvalence of IL viruses is rather meager, there do not seem to be any differences among the viruses. There do appear to be some differences in the clinical response of the host to the various viruses tested. The differences in gross pathologic change and the tissue response are not great.

The response of individual birds to any one virus is quite variable as indicated by (1) clinical signs (2) gross pathologic change and (3) the time and rate of cellular response. Within the scope of this experiment, the exact time of any tissue response cannot be determined. A large number

of similar tests would be necessary to obtain data in regard to the timing of these changes.

It has been shown in this experiment that the 146 virus will produce a sinus response which is characteristic of an infectious laryngotracheitis virus. This method of challenge has the advantage of the vent route in that the reaction is easier to read and is more clear cut.

Although tissue fixatives of a low pH gave the best results as far as bringing out the intranuclear inclusion bodies, the acid reaction does not produce the most brilliant and advantageous staining reaction of cytoplasm and nucleus when hemotoxylin and esoin are used.

Virus 1040 was isolated from a less acute case of IL and has not shown the acute pathogenicity of virus 742 and the commercial vaccine strains.

SUMMARY

Additional laboratory tests have indicated that 146 virus is that of infectious laryngotracheitis. There do not appear to be any differences in the antigenicity of the several viruses tested although there are minor differences in the clinical response of the host. Fixatives of low pH are most suitable for the demonstration of the intranuclear inclusion bodies of infectious laryngotracheitis in tissues stained with hematoxylin-eosin. The time and rate of cellular response to infectious laryngotracheitis virus vary from bird to bird.

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THE CLINICAL AND SEROLOGICAL RESPONSE OF CHICKENS TO CERTAIN LARYNGOTRACHEITIS VIRUSES

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THE isolation of a viral agent (146 virus) believed to be similar to the virus of infectious larygotracheitis (IL) of chickens was reported by Cover[2] in 1956 and by Cover and Benton[3] in 1957. This virus was relatively innocuous to chickens when administered via the respiratory tract but induced an immune response against commercial vaccine strains of IL viruses. Some question as to the true nature

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of this virus was raised by the failure of serum from chickens hyperimmunized with a commercial IL virus to neutralize the 146 virus. Subsequent to these reports comparative histopathological studies[4] with several strains of IL viruses have established a marked correlation in the tissue changes, including the presence of typical inclusion bodies in the tracheal epithelium, produced by the 146 virus and the more virulent IL strains. The 146 virus, therefore, is considered to be a less virulent strain of IL.

This report is concerned with the further investigation of the response of chickens to the 146 virus and the comparison of this response to that produced by more typical IL viruses.

MATERIALS AND METHODS

The 146 virus inocula used in the experiments consisted of either the chorio-allantoic fluids (CAF) or a broth suspension of chorio-allantoic membranes (CAM) harvested from embryonated chicken eggs previously inoculated with this virus. The CAM suspensions were prepared by grinding a weighed quantity of infected membrane with sterile sand in a sterile mortar and then suspending the ground tissue in either equal or five parts volume by weight of sterile nutrient broth. The resulting material was then centrifuged in a size 1 International centrifuge at 1500 rpm for 10 minutes and the supernatant was removed and saved. All such inocula were tested for freedom from bacteria and Newcastle disease virus and were stored in corked tubes at –15 C until used.

The IL viruses employed for comparative purposes or in challenge procedures were obtained either as commercial vaccines or were isolated in this laboratory from field cases. Two commercial vaccines were used and are designated as C-1 and C-2 in this report. The virus from a field case used in these experiments is designated 742 and was isolated from birds showing typical signs and lesions of IL and had previously exhibited virulence for chickens when administered via the respiratory tract.

In the administration of the 146 virus by the intranasal

route, one drop of the inocula was delivered from a 20 gauge needle into the anterior naris of each bird. When a virus inoculum was administered by atomization, a suitable spraying mechanism was used but varied among experiments and will be explained for each. All of the commercial vaccines which were used in vaccination procedures were administered in the usual manner by brushing the vaccine into the vent. When a virus was used for challenge by the intrasinus or intratracheal routes, 0.10 ml, 0.25 ml or 0.30 ml of the virus suspension was inoculated. For a vent challenge, the virus was brushed into the vent mucosa. The reactions to challenge were checked on the fourth or fifth day post inoculation. The presence of sinus swelling or nasal exudate was considered as a positive reaction to the intrasinus challenge. In the interpretation of the intratracheal challenge, the presence of exudate, blood, or a pseudo-membrane were classified as a positive reaction. The response to the cloacal challenge was considered positive if a typical "take" was in evidence.

The substation facilities located at Georgetown, Delaware, were used in those experiments designed for the evaluation of the response to the 146 virus under field conditions. The facility used was a standard type house divided into 20 pens with an area of 300 square feet each. Five wooden partitions completely divided an area on either side of a central feed room into five subdivisions each of which was further divided by a wire partition. Pen assignments were designed in such a way as to minimize the spread of the viruses used.

Titration of the various virus preparations was accomplished by inoculating either 0.1 ml or 0.2 ml of serial tenfold virus dilutions into the chorio-allantoic sac (CAS) of ten-day embryonated eggs using five eggs per dilution. Two mg of streptomycin and 2,000 units of penicillin were routinely added per ml of the serial dilutions. All embryos dying after the first day post inoculation and exhibiting typical plaque formation on the CAM or which survived to the 18th day of incubation but exhibited typical plaque formation

were considered as evidence of viral infectivity. Fifty percent embryo infective doses (EID_{50}) end points of titrations were calculated by the method of Reed and Muench.[7]

Serum neutralization tests were also performed by the inoculation of the CAS with 0.1 ml of a mixture of equal parts of the virus dilution and serum which had been allowed to incubate at room temperature for 30 minutes. Virus controls were mixed with equal parts of nutrient broth and five eggs were used for each dilution.

Intranasal administration. In order to investigate the immunity produced by the 146 virus when given via the intranasal route under field conditions, harvested CAF was administered to each of 800 four-week old Vantress X Arbor Acre chickens which were distributed equally among four pens located on one side of the feed room in the substation facility. At the same time a similar number of birds, distributed as above, in the other end of the same house were vaccinated in the usual manner with a commercial vaccine (C-1). Four pens of 200 birds each, in the same house, were not vaccinated and were held as unvaccinated controls. At four weeks post inoculation, 25 birds, randomly selected from each pen, were challenged by the intratracheal route using 0.25 ml of virus 742. A like number of birds from each pen were challenged by the cloacal route with the C-1 strain.

Administration by atomization. In order to ascertain the effect of administering the 146 virus by atomization and to compare this with results obtained with the same virus by the intranasal route as well as with a commercial vaccine employed in the usual manner, the following experiment was carried out using the substation facilities as above. Sixteen hundred 6-day-old Vantress X Arbor Acre chicks equally distributed among four pens were subjected to 7.5 ml of a CAM suspension per pen by atomization. This preparation had a EID_{50} of $10^{4.7}$ per ml and was administered from a commercial hand vaccine sprayer. These chicks had been vaccinated against Newcastle disease with the B-1 strain by the intraocular route on the previous day. Later at 16 days of age, the 146 virus was administered by spraying 7.5 ml

of a 1:100 dilution of the same CAM suspension over the heads of the birds in each of four pens as above. Also at this time a similar number of birds were vaccinated intranasally with 1:10 dilution of the same suspension and a like number were vaccinated by the vent method using strain C-1. A group of 1600 birds distributed as above were not vaccinated and were held as controls. When these birds were 10 weeks of age, 15 birds from each pen were challenged by the intratracheal administration of 0.25 ml of infected CAF of virus 742. Ten birds from each pen were challenged by the cloacal route with the same virus.

Influence of virus titer on reaction to atomization. Subsequent to the above experiment in which a rather severe reaction was noticed in those birds which received the 146 virus by atomization, an experiment was designed to ascertain the influence of virus concentration of the inoculum on this reaction. A CAM suspension of 146 virus was diluted with sterile nutrient broth so as to attain two virus concentrations with an EID_{50} of approximately $10^{4.7}$ per ml and $10^{3.7}$ per ml. Each of the above inocula were then administered in 7.5 ml amounts from a commercial hand vaccine sprayer to 400 5-week-old Vantress X Arbor Acre chickens in each of duplicate pens. Also at this time, the same virus suspensions were administered by the intranasal route to a like number of birds distributed as above. In addition, birds in duplicate pens were vaccinated by the vent method using strain C-1 and duplicate pens were held as non-vaccinated controls. Four weeks post vaccination 25 birds, randomly selected from each pen, were challenged by the intrasinus route with a CAM suspension of IL virus (742) and 25 birds from each pen were challenged by the vent method using the same virus.

Influence of age of vaccination on reaction to atomization. The influence of the age of vaccination on the reaction observed when the 146 virus was administered by atomization has been explored in two trials. In the first experiment, 15 4-week-old New Hampshire chicks and 15 10-week-old New Hampshire chicks were sprayed by use of an H and Z

hand sprayer with 4 ml of a 146 virus CAM suspension which has an EID_{50} titer of $10^{4.7}$ per ml. Subsequent to vaccination, the groups were held in separate batteries in the same isolation room and observed daily for four weeks. At the end of this period, all birds were challenged by the cloacal route with strain C-2. In the second experiment, 20 Delaware chicks 2½ weeks of age, 20 New Hampshire chickens 6 weeks of age, and 13 New Hampshire chickens 22 weeks of age were sprayed with 2 ml of CAM suspension of virus 146. This suspension had an EID_{50} of $10^{5.4}$ per ml and was administered from a No. 15 Devilbis atomizer over each group of birds in an enclosed area in which they were confined for a period of 10 minutes. These groups of birds were then held in separate batteries, with the younger birds in a heated unit, in one isolation room and observed daily for 14 days. At the end of this time, half of each group was challenged by the intrasinus route with 0.1 ml of a CAM suspension of the C-1 strain and half was challenged with 0.1 ml of a CAM suspension of the C-2 strain.

Onset of immunity. In order to investigate the onset of immunity following the intranasal administration of the 146 virus and to compare this with the onset of immunity produced by a standard vaccine administered in the usual manner, the following experiment was carried out. Thirty 8-week-old chickens of mixed breeding were given one drop of a CAM suspension of 146 virus with an EID_{50} $10^{5.4}$ per ml. Thirty chickens of the same age and breeding were vaccinated with a commercial vaccine (C-2) via the cloacal route. At intervals of 2, 4, 8, 12 and 21 days post vaccination six birds from each group were removed and three challenged via intratracheal inoculation while three were challenged by the intrasinus route using 0.3 ml of the C-1 strain. At each interval, unvaccinated susceptible birds were challenged by each route for comparative controls. Birds were examined for response to challenge on the 4th day following inoculation.

In order to ascertain the serological response of the birds in this experiment, serum was obtained from five birds of each group prior to vaccination and from those birds

challenged on the 12th and 21st day after vaccination. Subsequent serum neutralization indices against the respective vaccine virus were determined.

RESULTS

Immunity following intranasal administration. The birds which received the 146 virus exhibited no signs of respiratory distress other than a transient respiratory reaction which commenced on the 6th day post inoculation and subsided by the 8th day. Birds which received the commercial vaccine in the usual manner were checked for "takes" on the 4th post vaccinal day and exhibited a total response. The results of the challenge at 4 weeks post inoculation are given in table 1 and indicate that both groups of vaccinated birds exhibited an immune response while the non-vaccinated controls were mostly susceptible. The percentage of immune birds was similar for both vaccinated groups.

TABLE I

Results of challenge at 4 weeks post inoculation of birds vaccinated with 146 virus intranasally and commercial vaccine in vent

Vaccination	Method of Challenge			
	Intratracheal*		Vent**	
	Negative reaction	Positive reaction	Negative reaction	Positive reaction
146 Virus intranasal	63%	37%	42%	58%
Commercial vaccine-vent	62%	38%	31%	69%
No vaccination	15%	85%	2%	98%

* 0.25 ml. CAF of virus 742
** Commercial strain brushed into vent

Administration by atomization. A severe reaction to the 146 virus was observed in those birds vaccinated at 6 days of age by this method. This response was characterized by rales, wheezing, swollen and watery eyes, and mortality. It became apparent on the 4th day following vaccination, reached its peak on the 6th day, and had subsided almost completely by the 8th day after inoculation although a few birds continued to die from time to time. Dead birds exhibited principally a tracheitis with the trachea containing a thick yellowish mucus and slight cast formation. Definite

plugs and pseudo membranes were seen in only an occasional bird. During this reaction period, two pens suffered a 16 percent mortality in each; a third pen, 19 percent, and the fourth pen, 40 percent losses. Only a mild and transient reaction was observed in the 16-day-old birds atomized with a 1:100 dilution of the original suspension and in the same age birds vaccinated intranasally with a 1:10 dilution.

The results of the challenge procedures used in this experiment could not be interpreted due to the absence of an adequate response among the non-vaccinated birds and are not reported.

Influence of virus titer on the reaction to atomization. Chickens in those pens receiving a virus suspension containing $10^{4.7}$ EID_{50} per ml were adversely affected with the signs and lesions observed being essentially the same as described for the previous experiment. The reaction period commenced on the 6th day post vaccination and continued through the 8th day. Birds in those pens receiving a vaccine adjusted to a virus concentration of $10^{3.7}$ EID_{50} per ml experienced a less severe reaction than in the above pens, while those groups receiving the virus by intranasal instillation exhibited a still milder reaction. The results of these reactions are presented in table 2. Again the challenge results could not be interpreted and are not presented.

TABLE II

Influence of virus concentration and route of administration on reaction to 146 virus

Method of administration	EID_{50}/ml.	% mortality during reaction*
Atomization	$10^{4.7}$	22%
Intranasal	$10^{4.7}$	1.12%
Atomization	$10^{3.7}$	3.25%
Intranasal	$10^{3.7}$	0.00%

* Average of duplicate pens.

Influence of age of vaccination on reaction to administration by atomization. In the first experiment, signs of respiratory distress, i.e., rales, wheezing and a few swollen eyes were observed in the 4-week age group on the 4th day after administration. One bird died from this group on the

4th and two died on the fifth day following atomization. Autopsy of the dead birds revealed a hemorrhagic tracheitis with the presence of yellow exudate and a slight film formation. The eyelids of these birds were extensively inflamed. The rather severe respiratory signs persisted until the 7th day post vaccination in this group. The 10-week-old group exhibited no mortality and only a mild and transient reaction commencing on the 5th day post vaccination and subsiding on the 7th day. The subsequent challenge one month later revealed all remaining birds of both groups to be immune.

In the second experiment, signs of generalized respiratory distress were observed on the 4th day post vaccination in the 2-week-old and 6-week-old groups. The signs in the 2-week-old group consisted of gasping, wheezing, and swollen, watery eyes with some completely closed. One bird died in the 2-week-old group on the 6th day post vaccination and exhibited only clear mucus in the trachea. In the 6-week-old group, two birds exhibited watery eyes and the respiratory signs were relatively mild. In the 22-week-old group, generalized signs were not apparent; however, one bird exhibited marked dypsnea and died on the 5th day post vaccination and another died on the 14th post vaccinal day. Both of these birds exhibited a severe inflammatory reaction in the trachea with the presence of considerable whitish to yellow exudate but did not exhibit plugs, pseudomembrane formation or free blood. The signs subsided by the 8th post vaccinal day in the two younger groups. The results of the challenge two weeks after vaccination showed that all birds were immune with the exception of one bird from the 2-week-old group which was susceptible to strain C-2. This bird, however, did not exhibit the marked reaction observed in the non-vaccinated controls. These results are given in table 3.

Onset of immunity. Vaccinated birds challenged on the 4th day after inoculation exhibited a milder reaction than did the susceptible controls, but nevertheless were classified as positive. Some protection was apparently present on the 2nd and 4th days after vaccination but was most pronounced

at the challenge at 8 days. One bird from the C-2 vaccinated group was susceptible on the 8th day and another from the same group was susceptible on the 21st day following vaccination. These results are shown in table 4. The results of serum neutralization tests are shown in table 5. The neutralization indices of sera obtained on the 12th and 21st days post vaccination indicate a general increase over those of the pre-vaccination samples. However, serum from individual birds failed to exhibit an appreciable neutralization index even though immune to challenge.

TABLE III

Results of intrasinus challenge at 2 weeks post vaccination of several age groups which received 146 virus by atomization

Age of birds when vaccinated	Strain of challenge virus*	
	C-1	C-2
2½ weeks	0/8**	1/10
8 weeks	0/10	0/10
22 weeks	0/5	0/6
Controls***	3/3	3/3
Controls****	4/4	4/4

* 0.1 ml. injected.
** Number positive/number challenged.
*** Same age as youngest age group when challenged.
**** Same age as intermediate age group when challenged.

TABLE V

Serum neutralization indices of sera* against homologous virus prior to and at intervals after vaccination with 146 virus and C-2 virus

Vaccine virus	— Neutralization Index —		
	Pre-vaccination	12 days Post-vaccination	21 days Post-vaccination
146 Virus	0.68	1.62	1.51
	0.18	0.95	0.68
	1.18	1.82	1.05
	0.28	1.28	1.68
	0.20	0.45	1.25
	——	——	1.75
C-2 Virus	0.87	1.51	0.67
	0.33	0.68	0.00**
	0.35	1.05	2.75
	0.54	1.68	0.84
	0.00	1.25	1.17
	——	1.75	——

* Serum samples taken from different birds at each interval.
** Positive reactor on intrasinus challenge.

TABLE IV

Results of challenge at intervals after vaccination with 146 virus and commercial strain (C-2)

Vaccine used	— Days after vaccination —									
	2nd		4th		8th		12th		21st	
	Trx.	Sinus	Trx.	Sinus	Trx.	Sinus	Trx.	Sinus	Trx.	Sinus
146 IN	3/3*	2/3	3/3	3/3	0/3	0/3	0/3	0/3	0/3	0/3
C-2 in vent	3/3	2/3	2/3	2/3	0/3	1/3	0/3	0/3	0/3	1/2
Controls	2/2	2/2	2/2	2/2	3/3	3/3	3/3	2/2	1/3	3/3

* Denotes number positive/number challenged.

DISCUSSION

Although it would have been desirable to have obtained a higher percentage of positive reactors to challenge among the non-vaccinated birds in the first field trial reported, the results in this test appear to compare favorably with the protection afforded by the commercial vaccine administered in the usual manner. The age of the birds when the vaccines were administered must be considered since the size of the vent at this time may exert some influence on the efficacy of the vent method of vaccination. Beaudette[1] reported in his early work that the most desirable age for vaccination by the vent method is between 2 and 3 months. In subsequent battery type expirements, however, vaccination of 8-week-old birds with both type viruses has indicated that the methods are comparable. However, our experiments did not establish the degree of immunity, so that the strength of the immunity produced needs further clarification.

Rather unfavorable results were obtained when the 146 virus of too high a concentration was administered by atomization in contrast to the mild reaction observed following intranasal administration. A similar experience with the B-1 Newcastle virus was reported by Johnson[6] in his work with nebulization procedures. Whether the lower virus concentration, required to eliminate the undesirable reaction, will be sufficient to induce adequate immunity remains to be seen.

Our results concerning the rapidity of the onset of immunity following vaccination for IL are similar to those previously reported by Hitchner[5] and further indicate that the immunity induced by the 146 virus parallels that produced by a regular IL virus. The duration of such immunity will require further investigation.

The results of the experiments designed to investigate the influence of age of vaccination on the reaction following atomization were somewhat confusing. It would appear that the younger birds in each experiment were more adversely affected than the older birds. However, the death of two adult birds in one of these experiments does not support this line

of thought. The fact that a source of heat was available for the young group of birds in the second experiment may account for the lower mortality as compared to the young birds of the first experiment. Further investigation and the consideration of other factors such as parental immunity are needed to clarify this point.

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SUMMARY

The response of chickens to the 146 virus, a less virulent strain of infectious laryngotracheitis virus, when administered via the respiratory tract was investigated under experimental and field conditions. The influence of the method of administration and age of bird on this response were also studied. The results of these experiments indicate:

1. That the 146 virus given via the intranasal drop method appears to induce an immune response comparable to that produced by the usual vaccination procedure for infectious laryngotracheitis.

2. That the administration of the 146 virus by atomization produces an adverse reaction characterized by acute respiratory distress and mortality and that this reaction may be influenced by the virus concentration of the inoculum used and to some extent, by the age of the bird when vaccinated.

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SALMONELLA ORGANISMS ISOLATED FROM POULTRY FEED*

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THE finding of *Salmonella* organisms in poultry feeds, animal feeds, or feed ingredients has been reported by a number of workers (Griffin;[6] Galton, Harless, and Hardy;[5] Erwin;[4] and Thal, Rutqvist, and Holmqvist[8]). It is the purpose of this paper to describe an outbreak of salmonellosis in experimental poults in which the serotype isolated from the poults was thought to have been present in commercial turkey starter mash.

CASE HISTORY

Fifty-one day-old poults hatched January 8, 1957 in a commercial hatchery were received via car and handled as follows: 36 were placed immediately in two decks (18 in

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each) of a hen-battery brooder in a thoroughly clean room of an animal-disease isolation building. Nine of these were dead by the end of the first week. The remaining 15 out of the group of 51 were divided into three lots of five each and placed in three small sterilized wire-floored and heated cages in another building. The poults in one of these three cages were maintained as uninoculated controls, but all five died by the 7th day. The only lesion found on postmortem examination of the nine poults that died by the end of the 1st week and of the five poults that died in the control cage was unabsorbed yolk sacs. Bacteriological cultures inoculated from the liver and intestines revealed *Salmonella thomasville*. Poults from the same hatch raised on other farms had suffered no morbidity nor mortality. In view of this information, we decided to examine the feed. Sterile pint jars were used to take samples of feed from the containers in each of the involved rooms. One-gram samples were placed in each of the six tetrathionate and six selenite F broth tubes. After 18 hours of incubation, inoculum from the enrichment-broth tubes was seeded on brilliant-green-agar plates. From these, suspicious salmonellae colonies were fished to triple-sugar-iron-agar and to urea-agar slants. Cultures that showed typical salmonellae reactions were then transferred to carbohydrate media and subjected to antigenic analysis.

RESULTS

No *Salmonella* organisms were isolated from the two initial feed samples. Several days later two more feed samples were collected from an unopened sack of turkey feed in our feed house. It is believed that this sack of feed belonged to the same batch as that being fed to the poults. *S. thomasville* was isolated from two of the six tetrathionate tubes inoculated from one of the two samples. This was the first time that this serotype was identified in our laboratories.

For several years we had been working on a transmissible enteritis of turkeys and using oral inoculations of in-

testinal suspensions from suspected cases in propagating the disease. Because this type of enteritis was distinct from *Salmonella* infection, it was necessary to maintain inoculum free from these organisms in order to obtain a true picture of our experimental disease. From time to time we encountered morbidity and occasionally mortality in some of our uninoculated experimental controls. Frequently salmonellae were isolated from the dead birds. This would sometimes occur even though bacteriological examinations of the poults before use in our experiments failed to yield salmonellae. After isolation of *S. thomasville* from the poults and then from the turkey feed, we began to test other samples of feed for evidence of these organisms. Thirty-three unopened samples of turkey-starter mash and seven of turkey grower or breeder feed were examined bacteriologically. From five of the starter-mash samples six *Salmonella* serotypes were isolated. They were *S. thomasville, S. tenessee, S. cubana, S. kentucky, S. bareilly,* and *S. thompson.* Four samples of fish meal, nine of chick starter or grower, and two samples of duck grower all were negative for *Salmonella.*

Twenty-one samples of meat scraps and meat-scrap ingredients were obtained in sterile pint jars during visits to four rendering plants. Samples were collected in each plant from the various stages in the manufacture of this product and from the raw materials used. The latter consisted of bone, poultry, pork, beef, and lamb offal from slaughter houses, butcher shops, and hotels. There were five samples of the finished product called "50% meat scraps" and three of these were positive for salmonellae. One contained *S. senftenberg,* another *S. tennessee,* and a third both *S. illinois* and *S. montevideo.* An intermediate product called "Doo Meal" was collected along with the third sample. It had been cooked and the fat extracted with naphtha but it was not ground and blended. It yielded *S. illinois.*

In the preparation of meat scrap, the bone and offal is dumped directly from the delivery trucks into a course grinder and then goes into the cooker. The cooker consists of very large, double-jacketed boilers. Steam is put into the outer

jacket while the inner vat slowly turns. The material is held at 250 F until the moisture content of the mass is reduced to 5 percent. The heating period varies with each batch depending on the amount and type of offal. The first samples collected in the plants were taken directly from the cookers or just after removal from them. In some cases they were still hot or warm. These cooked samples were called unpressed and unextracted cracklings. They consisted of cooked offal and chunks of bone. All of these samples were negative for salmonellae. Four to six samples were collected in each plant, taken in sequence during the processing of the finished product. After the first set of samples was taken from the cooker practically all of the other samples were collected from open piles on warehouse floors or from moving belts going from one processing step to the next.

In view of the foregoing, we decided to make postmortem and bacteriological studies of all groups of poults purchased from hatcheries. This was done immediately upon arrival of the birds. If no dead birds were found in the boxes, we killed four to six out of each hundred in order to make these tests. The livers, yolks, and intestines were put through selenite F and tetrathionate broths as previously described. Batches found to be free of salmonellae were placed in isolation units and all dead birds or feces checked at weekly intervals thereafter. Ten groups were studied in this manner. Five were negative for salmonellae on arrival and remained so. The other five were negative on arrival but *S. newbrunswick, S. kentucky, S. bredeney, S. anatum, S. cubana,* and *S. saintpaul* were isolated later from their feces.

Observations and examinations carried out on commercial poults purchased for experimental use show that the following *Salmonella* serotypes were isolated from 20 of 63 groups raised under partially isolated conditions. These were not raised in an animal disease isolation building like the previous ten groups just described but rather were placed at once in a steam sterilized battery kept in a freshly cleaned and fumigated room and cared for by one person. They were not checked on arrival for the presence of salmonellae. The

serotypes isolated were *S. saintpaul, S. derby, S. bredeney, S. montevideo, S. bareilly, S. kentucky, S. panama, S. anatum, S. lexington, S. newbrunswick, S. binza, S. minneapolis, S. thomasville, S. senftenberg, S. cubana,* and *S. minnesota.*

DISCUSSION

From the data presented, there is no clear-cut proof that the *S. thomasville* which caused a mortality of 34 percent in our poults came from the feed eaten by the poults at the time of its isolation. However, the recovery of this serotype from an unopened sack that was believed to be from the same batch of feed coupled with the fact that this was the first time such a serotype was even isolated in this area, is certainly strong circumstantial evidence that the feed was the source of the infection.

Data from the ten groups of poults tested and found free of salmonellae on arrival showed that 50 percent had positive feces by the 3rd week. The presence of salmonellae in the feces without morbidity in experimental poults still poses confusing problems of correct interpretation of results. There is evidence to show that salmonellae in the feces of young poults may, under certain adverse conditions, cause a septicemic outbreak at any time.

It should be emphasized that all salmonellae isolated from the samples collected in the rendering plants were from the material that had been cooked previously and stored in open piles on warehouse floors. Based on our findings, it seems reasonable to assume that the cooking process does kill the *Salmonella* organisms in the material, but the products become contaminated during subsequent handling and storage. There was ample opportunity for rats, mice, cats, pigeons, and wild birds to contaminate the piles of meat scrap ingredients. To prevent the contamination of poultry feeds with salmonellae in meat scraps, better methods of storage must be employed. Serotypes that we recovered from feed are known to be capable of causing outbreaks not only in turkeys but also in humans (Hinshaw and McNeil,[7] Bruner,[1] and Clarenburg[8]).

According to Buxton,[2] carrier animals, primarily poultry and pigs, serve as the main reservoir of infection and the development of a fatal *Salmonella* bacteremia is dependent upon a variety of factors namely the age and general well being of the host. Certainly, feeding *Salmonella* organisms to poultry seriously limits the effectiveness of testing programs for the control of paratyphoid diseases.

SUMMARY

A case of high-poult mortality from a bacteremia caused by *Salmonella thomasville* is described.

Isolation of nine serotypes from turkey feed and meat scraps were as follows: *S. thomasville, S. tennessee, S. cubana, S. kentucky, S. bareilly, S. thompson, S. senftenberg, S. illinois,* and *S. montevideo.*

The isolation of 16 serotypes from the feces of experimental poults is described.

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RESPIRATORY FLORA AND DISEASES OF ANTARCTIC BIRDS*

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LITTLE has been done on the respiratory flora and diseases of Antarctic birds. Bunt[2] reported a high incidence of nasal infections in Antarctic birds at Macquarie Island and found Gram positive organisms in nasal smears. Aspergillosis[15] and salmonellosis[3] have been reported in penguins kept in captivity. Ornithosis has been found in oceanic birds[6,8]. During the 1957-58 Argentine Antarctic Expedition through the South American Quadrant of Antarctica, microbiological studies on Antarctic birds were conducted in a temporary laboratory aboard the icebreaker ARA "General San Martin"[12]. The purpose of this note is to present results on serological tests for salmonellosis and ornithosis and observations on the respiratory flora.

METHODS AND MATERIALS

Blood for serum was obtained by cardiac puncture immediately after shooting the flying birds and before sacrificing the penguins by cervical dislocation. The serum from the clotted blood was preserved with Merthiolate† and refrigerated. *Salmonella* antibodies were detected by the indirect hemagglutination test (Sieburth[10,11,13]). The serums were screened with a polyvalent preparation containing antigens to serogroups B, C_1, C_2, D and E_1. Samples having a titer of 1:80 or above were tested with monovalent preparations.

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† Thimerosal, Eli Lilly

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Antibodies to the ornithosis virus were detected by the direct complement fixation test (Brumfield and Pomeroy[1]). Five percent normal chicken serum was added to the antigen mixture. The psittacosis antigen was titrated with four units of immune avian serum.

The birds were opened as aseptically as possible and tracheal swabs were put on Eugon agar (BBL) slants. Liver and intestinal material were inoculated into tetrathionate broth (Difco) for salmonella enrichment. The tetrathionate cultures were streaked on SS agar (Difco), suspicious colonies were picked to Eugon agar slants and the slide agglutination test was performed with polyvalent salmonella antiserum.

RESULTS

The birds identified by number (table I) were obtained at the following locations: Ushuaia, Tierra del Fuego Island (1); Half Moon Island, South Shetland Islands (3, 4, 5, 6, 7, 14, 17, 18, 19); Almirante Brown Base (15) and Hope Bay (8, 9, 10, 11, 12, 20) on Palmer Penisula; Laurie Island, South Shetland Islands (21, 22, 28); Southern Thule Island (23) and Zavodovski Island (26, 27) in the South Sandwich Islands; and Halley Bay (25) in the Weddell Sea. The birds were obtained at the peak of the Antarctic summer between December 5, 1957 and February 5, 1958.

None of the 17 birds examined had gross lesions in the lungs or air sacs. In an attempt to detect a respiratory flora, tracheal swabs from six penguins and four flying birds were cultured and the results are given in table 1. The tracheas of all six penguins were free of mucus, lesions and a detectable aerobic flora. In contrast all four flying birds exhibited a tracheitis with mucous secretions and petechial hemorrhages. An abundant tracheal flora was obtained from three of these four birds. *Proteus vulgaris* and an anaerogenic *Escherichia intermedia* were isolated from skua gull 1. *Escherichia coli* was obtained from skua gull 26 and sheathbill 28. The *E. coli* isolates were tested with anti-serums to seven serological types found in chronic respiratory disease of chickens and were found to be serologically unrelated. All

four cultures were tested for pathogenicity in chickens using the air sac inoculation procedure of Gross [5]. The left smaller abdominal air sac of five seven-week-old chickens was inoculated with 0.2 ml of an 18 hour broth culture. The heart, trachea and air sacs were examined 3 days post-inoculation. All four cultures caused similar invasive lesions in the air sacs and were considered mildly pathogenic. Slide agglutination tests with pleuropneumonia-like organsims of avian origin were performed with serums from seven penguins and four flying birds. No antibodies to this organism were detected.

TABLE I

Respiratory flora and lesions in Antarctic birds

Bird No.	Species	Tracheal lesions	Tracheal cultures	
			Species	Lesions in chickens*
20	Adelie penguin	—	—	
22	Adelie penguin	—	—	
21	Ringed penguin	—	—	
23	Ringed penguin	—	—	
15	Gentoo penguin	—	—	
25	Emperor penguin	—	—	
1	Skua gull	+	*Escherichia intermedia*	Pathogenic
			Proteus vulgaris	Pathogenic
26	Skua gull	+	*Escherichia coli*	Pathogenic
27	Giant fulmar	+	—	
28	Sheath-bill	+	*Escherichia coli*	Pathogenic

* Ability of 18 hour broth cultures to cause invasive lesions in inoculated air sac of seven week old chickens (5 per culture).

The results on the serological tests for *Salmonella* and ornithosis antibodies are shown in table 2. The polyvalent *salmonella* indirect hemagglutination test indicated, that of the 24 serums tested, 11 were negative, 9 had non-specific titers (1:40 or less), 3 were suspicious (1:80) and 1 was positive (1:160 or higher) on the basis of titers in chickens. Suspicious titers were given by skua gull 1 to the E_1 antigen and by sheath-bill 11 to the C_2 antigen. Positive titers to the E_1 antigen were given by ringed penguin 21 and sheath-bill 3. No *Salmonella* isolations were obtained from any of the 17 birds cultured.

Twenty of the serums were suitable for the direct complement fixation test for ornithosis antibodies. The serum from gentoo penguin 7 was somewhat anticomplementary and gave a suspicious reaction. All three serums from the sheath-bills gave positive reactions.

TABLE II

The incidence of salmonellosis and ornithosis in Antarctic birds as indicated by serological tests

Species and number	Salmonella I HA Test[1]		Ornithosis CF test[2]
	Polyvalent	Monovalent	
Adelie penguin			
5	—		—
9	1:10		—
10	—		—
12	—		—
20	1:40		ND
22	—		ND
Emperor penguin			
25	—		—
Gentoo penguin			
6	1:10		ND
7	1:10		1:4
14	—		—
15	1:10		—
18	1:20		—
Ringed penguin			
21	1:80	1:160-E_1	ND
23	—		—
Skua Gull			
1	1:80	1:80-E_1	—
4	—		—
8	1:10		—
17	—		—
19	1:20		—
26	—		—
Giant fulmar			
27	—		—
Sheath-bill			
3	1:160	1:320-E_1	1:16
11	1:80	1:80-C_2	1:8
28	1:20		1:16

[1] In domestic birds 1:80 is suspicious and 1:160 is considered positive
[2] In birds from temperate zones 1:8 and higher are considered positive

DISCUSSION

A high percentage of chickens react serologically to pleuropneumonia-like organisms which are considered the primary etiological agent of chronic respiratory disease of

chickens (Jungherr *et al.*,[7] 1955). Slide agglutination tests on 11 serums from Antarctic birds failed to detect antibodies to pleuropneumonia-like organisms of avian origin. However, *Escherichia coli*, which is often found as a complicating factor in chronic respiratory disease of chickens (Gross[4]), was present in the tracheas of a sheath-bill and a skua gull possessing tracheal lesions. The isolation of mildly pathogenic Gram negative rods from three of four flying birds obtained at widely separated areas, indicated that this condition may be quite common. Although penguins are susceptible to respiratory diseases in captivity (Halloran[7], Tilden *et al.*[15]), those studied were free of tracheal lesions and a tracheal flora. The absence of a tracheal[1] flora may be due in part to the extremely low bacterial content of the Antarctic air (less than one organism per 25 liters) and the presence of strong antibacterial substances in the diet of the penguins studied (Sieburth[12]). Although nasal infections were not observed, Bunt[2] found a high incidence of nasal infections associated with Gram positive organisms in Antarctic birds on Macquarie Island.

The number of bird carcasses found shot and dissected at the various bases indicated that a number of Antarctic birds are used for game and amateur taxidermy. This intimate contact with birdlife might be a source of human infection. Serological data indicated that both salmonellosis and ornithosis probably occur in Antarctic birds. All three sheath-bills obtained at the bases in the Antarctic Arc had complement fixing antibodies to the ornithosis virus. Ornithosis has apparently not been reported in the Sheath-bill (Halloran[7]). However, the pigeon-like sheath-bill migrates to the tip of South America where ornithosis is quite prevalent (Meyer[9]). This disease has also been reported in two oceanic birds, the Fulmar[8] and the Storm Petrel[6]. Antibodies to two *Salmonella* serotypes were found in a ringed penguin, a skua gull and two-sheath-bills; however, no *Salmonella* isolations were obtained. *Salmonella* species which are ubiquitous and have a low host specificity have been found in the herring gull (Steiniger and Hahn[14]) and in African penguins held in captivity (Cockburn[3]). The existence of birds in the Antarctic

with an apparent history of avian diseases transmissible to man, may be a health menace to the small Antarctic bases and refuges where men are isolated in small groups without a medical officer.

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SUMMARY

1. A tracheitis was observed in flying birds but not in penguins. The *Escherichia coli, Escherichia intermedia* and *Proteus vulgaris* tracheal isolates were mildly pathogenic for chickens.

2. Antibodies to pleuropneumonia-like organisms were not detected.

3. No *Salmonella* isolations were obtained from the livers or intestines of the seventeen birds cultured.

4. Serological data on twenty-four birds sugested a history of ornithosis in three sheath-bills and salmonellosis in a ringed penguin, skua gull and two sheath-bills.

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A REEVALUATION OF THE USE OF MEDIA FOR THE ISOLATION OF PLEUROPNEUMONIA-LIKE ORGANISMS OF AVIAN ORIGIN *†

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MANY workers [3,5,6,7,9,12] have described the isolation of pleuropneumonia-like organisms (PPLO) from the respiratory tract of birds. Other workers[1,4,8,10,11] have presented and compared isolation techniques. However, all of these studies must be reevaluated in light of the findings presented by Adler, Fabricant, Yamamoto and Berg[2]. The latter workers demonstrated that there was a wide variation in characteristics among PPLO of avian origin and that this variation was a major problem not only in the isolation of PPLO, but also in the interpretation of their pathological significance. Although five different serological types of PPLO were encountered in this study, so far only two types seem to be widely distributed and present a significant problem.

The first type, called S_6, is the standard antigen strain. This type is definitely pathogenic, makes a good antigen, and is characterized by a relatively slow growth rate in culture media. The second type, called C, is either non-pathogenic or weakly pathogenic, usually makes a poor antigen, and is characterized by a very rapid growth rate in culture media. These two types are completely unrelated serologically, there being no cross-agglutination between them.

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As pointed out by Adler et al.[2], the diagnostic problem was further intensified by the fact that birds affected with chronic respiratory disease (CRD) often yielded mixtures of these two types of organisms or what was more confusing, only the C or "non-pathogenic" type. In other cases of CRD, isolation attempts failed or were made only with great difficulty in spite of the fact that exudates from the original material were infectious for turkey poults. These findings suggested that the S_6 "pathogenic" type was often more difficult to isolate than the C type in culture media.

The purpose of this paper was to test a series of stock cultures of PPLO already on hand at this laboratory to see if similar types were present in this area (New York) and to test the use of the chick embryo and an enrichment medium for the isolation of avian PPLO.

MATERIALS AND METHODS

Chick embryos. The chick embryos used in this study came from a closed flock kept at the poultry disease experimental farm. Large samples of embryos, pip and cull chicks from this flock had been examined, cultured and found free of PPLO. No PPLO antibodies were found in any birds tested from this flock. Large numbers of fresh and incubated eggs were cultured and found free of PPLO.

Adler's blood slope overlay medium. This medium consisted of a blood slope overlaid with broth. The slope was prepared from bacto blood agar base (Difco) containing five percent fresh defibrinated horse blood. To this agar slope was added enrichment broth consisting of bacto PPLO broth (without crystal violet) to which was added ten percent horse serum, penicillin (1000 units per ml.) and thallium acetate (1:2000 concentration.)

PPLO agar medium. This medium consisted of bacto PPLO agar (Difco) to which was added ten percent horse serum and inhibitors as in the previous medium as well as one percent yeast hydrolysate (Nutritional Biochem. Co.).

Modified Grumble's medium. This consisted of Phenol Red Broth Base to which was added carbohydrate, serum

fraction and inhibitors as described in a previous paper[10].

The isolation techniques used in these experiments were essentially similar. Equal quantities of inoculum (0.1 ml.) were used by each of the following procedures:

a. Direct inoculation on PPLO agar plate.
b. Direct inoculation into modified Grumble's media—dextrose and lactose.
c. Inoculation into Adler's overlay medium and after three days of incubation, subculture to another tube of overlay medium and to PPLO agar and dextrose. Cultivation in Adler's overlay carried for three serial passages with subcultures to plate and dextrose each time if necessary.
d. Inoculation of six 6-day chick embryos into the yolk sac. If an embryo died before the 10th day after inoculation, yolk was harvested and subjected to procedures a, b and c. If no embryo deaths occurred, an embryo yolk was harvested at random and treated similarly.

A culture was not considered positive until colonies had been produced on agar plates and their growth rate observed. The criteria for evaluating the growth rate and the staining procedures used for organisms and colonies are described in a previous paper.[2]

RESULTS

1. Reisolation of PPLO from frozen embryo yolk culture.

Attempts were made to reisolate PPLO from yolk cultures of these organisms which had been stored in a mechanical freezer at —25 C for periods of 6 months to 6½ years. In the first experiment, eight isolations of PPLO were made from thirteen yolk cultures. Two of these eight isolations were C type. These two strains were readily isolated directly on PPLO agar and Grumble's media as well as after embryo passage. The other six isolates were S_6 type. None of these strains were isolated directly on culture media but only after embryo passage. Unfortunately, the direct Adler overlay medium series was lost in this experiment so we were unable to compare this series with those following.

There were three more similar experiments. Since all of these were carried out under identical conditions and with the same materials and methods they will be discussed as one group. In these experiments 33 isolations of PPLO were made from 47 yolk cultures. Fifteen of these isolations were C type and originated from 13 different strains. Eighteen of these isolations were S_6 type and originated from 12 different strains. Two of the original yolk culture strains yielded both C and S_6 type organisms.

Some of the results of this experiment are summarized in table 1. Here it is evident that there were differences in the relative effectiveness of the various isolation procedures tested. These differences were related not only to the isolation procedures used but also to the type of PPLO isolated. In general, both of the direct inoculation procedures gave poor results. The use of Adler's overlay as an enrichment procedure gave very good results with C type PPLO but only moderate success with S_6 type strains. The addition of a chick embryo passage to the procedure greatly increased the number of S_6 type isolations.

Two apparent discrepancies should be explained. The failure to isolate any of the 18 S_6 type strains when inoculated into chick embryos and subcultured to PPLO agar plates was probably due to the fact that the use of yolk as an inoculum on the plates left a film of fat droplets that tended to obscure the tiny colonies produced by this PPLO type. The isolation of only six of eleven S_6 type strains when inoculated into chick embryos, subcultured to Adler's and thence to dextrose was due to the fact that the recording of this column was halted after three blind passages in Adler's medium. All of these strains when carried through further passages were found capable of fermenting this carbohydrate.

The data in table 1 was rearranged to evaluate the significance of the chick embryo passage in the results. It was then found that 14 of the 15 C type cultures were reisolated in media without the need for embryo passage. Only one additional C type culture was isolated after embryo passage. All of the 13 C type cultures put through embryo

passage were reisolated. On the other hand, only seven of eighteen S_6 type cultures were reisolated in media. Six of these seven cultures were also reisolated after embryo passage. The other eleven cultures in this series were re-isolated only after passage through chick embryos.

TABLE I

A comparison of the effectiveness of various cultural procedures in the reisolation of PPLO from frozen embryo yolk cultures

Culture procedure	Type C	Type S_6
Direct inoculation in modified Grumble's dextrose	2/15[a]	1/18
Direct inoculation on PPLO agar plate	0/7	0/9
Inoculation into Adler's overlay medium for at least 3 passages, subcultured to modified Grumble's dextrose	14/15	3/17 (1)[b]
Inoculation into Adler's overlay medium for at least 3 passages, subcultured to PPLO agar plate	14/15	6/18
Inoculation of chick embryos, subcultured directly to modified Grumble's dextrose	12/13 (2)	9/18
Inoculation of chick embryos, subcultured directly to PPLO agar plate	13/13 (2)	0/18
Inoculation of chick embryos, subcultured through 3 passages on Adler's overlay, each overlay subcultured to modified Grumble's dextrose	4/4 (11)	6/11 (7)
Inoculation of chick embryos, subcultured through 3 passages on Adler's overlay, each overlay subcultured to PPLO agar plate	4/4 (11)	17/18

[a] In each fraction the numerator is the number of isolations by this method, the denominator is the total number of isolations made by all methods.

[b] The numbers in parentheses refer to number of successful isolations secured sooner by simpler techniques.

2. Reisolation of PPLO from dilutions of known S_6 type strains (comparative titrations).

A second series of experiments was designed to test the results just described. Twelve comparative titration trials were made with three different strains of PPLO, all of which were of the S_6 type. These strains had been grown for 35 or more passages in culture media and were well adapted to these media. In each trial, a series of decimal

dilutions of the culture to be tested as made in PPLO broth blanks. From each dilution tube of culture, subcultures were made by the following methods: (a) Direct inoculation of PPLO agar plate. (b) Direct inoculation of modified Grumble's dextrose. (c) Inoculation of Adler's overlay, subculture to plate and dextrose. (d) Inoculation of chick embryos. In these experiments a modification was made in that the criteria for the growth of PPLO in chick embryos were either the presence of lesions at the time of hatching, growth on subculture media, or both.

The results of these trials are shown in table 2. In six out of 12 trials, the titers in embryos were higher than those in media; in five out of 12 trials, the titers were equal; in only one out of 12 trials was the titer in media higher. In several trials, PPLO were grown in embryos from PPLO suspensions 100 to 1000 times more dilute than those from which growth occurred in media.

On three occasions, Adler's procedure gave titers higher than direct inoculation of Grumble's medium. The two procedures gave the same titer eight times and the Grumble's medium gave a higher titer once. In every case, the direct inoculation of PPLO agar plates gave significantly poorer results than any other method.

3. Comparison of techniques for isolation of PPLO from avian tissues.

A small number of comparative isolation attempts from tissues were made in a manner similar to those used in the previous two experiments. Fifteen of 15 C type cultures were isolated directly in media as well as after embryo passage while both of two S_6 type strains were isolated only after embryo passage. Continuation of these experiments with tissues from field cases of respiratory disease is planned.

DISCUSSION

This series of observations and experiments arose out of attempts to reactivate various PPLO strains in order to study their characteristics. After a group of reisolations were made and the strains classified on the basis of their

TABLE II

Comparative titrations of S. type PPLO strains by different cultural techniques. The results in this table are expressed as the log of the greatest tenfold dilution from which reisolation was successful.

Experiment No.	1	2	3	4	5	6	7	8	9	10	11	12
Strain	293	VB	293	293	293	293	VB	CA	293	293	CA	CA
Direct inoculation on PPLO agar plate	4	3	5	2	3	4	2	4	3	3	1	6
Direct inoculation of modified Grumble's dextrose	6	5	7	5	6	7	7	8	7	6	3	8
Inoculation of Adler's overlay, subculture to PPLO agar plate	7	7	8	5	6	8	6	8	7	6	3	8
Inoculation of Adler's overlay, subculture to Grumble's dextrose	7	7	8	5	6	8	6	8	7	6	3	8
Inoculation of chick embryos	9	9	9	5	9	7	7	8	7	7	5	8

Different experiments using the same strain of PPLO are not comparable since different age cultures were used as the starting inoculum.

growth characteristics into the C and S_6 types[2], it became apparent that these two types of PPLO varied greatly in the way they were affected by cultivation in the yolk sac of chick embryos. Embryo culture had little or no stimulatory effect on C type PPLO but a very marked stimulatory effect on S_6 type PPLO. This effect was confirmed by the data secured in the comparative titration experiments.

Since the presently available evidence indicates that it is the S_6 type strains rather than the C type strains that are pathogenic and associated with disease processes in chickens and turkeys, these data suggest that the media isolation procedures generally used for the isolation of PPLO and the diagnosis of CRD in these birds are not satisfactory. Some of the other reasons why our present PPLO culture media are unsatisfactory have been discussed in a previous paper.[2]

In spite of this dissatisfaction with our present media and procedures it can hardly be recommended that we go back to the routine use of chick embryos for the isolation of avian PPLO. It would not be practical because of the difficulty of securing embryo sources free of PPLO. However, these results should alert us to the need of developing better culture media and the possibility that some substance present in chick embryo yolk sac might be a vital component of such an improved PPLO culture medium.

SUMMARY AND CONCLUSIONS

1. Thirty-three strains of PPLO were reisolated from 47 yolk cultures that had been frozen at —25 C. for from 6 months to 6½ years. Fifteen of these strains were C type and 18 were S_6 type.

2. Fourteen of the 15 C type PPLO were reisolated in media without embryo passage. Only seven of the 18 S_6 type strains were reisolated in media. The other 11 strains in this series were reisolated only after embryo passage.

3. In a series of 12 trials in which isolations were attempted from serial dilutions of media-adapted S_6 strains of PPLO, again embryo passage was a more effective procedure than culture media for growing and detecting these organisms.

4. In view of the evidence that the S_6 types of PPLO rather than the C types are the pathogenic strains, these data suggest that the isolation procedures in media generally in use are not satisfactory.

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A *STREPTOBACILLUS*, THE CAUSE OF TENDON-SHEATH INFECTION IN TURKEYS*

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REPORTS of synovitis and arthritis in turkeys caused by bacterial and viral organisms occur in the literature. Jungherr,[9] Hinshaw and McNeil,[8] Fahey,[5] and Madsen[12] reported that such cases were caused by a *Staphylococcus;* Beaudette[1] mentioned an arthritis in turkeys due to *Erysipelothrix insidiosa (rhusiopathiae);* Higgins *et al.*[7] and Chaplin and Hamilton[4] reported salmonellae to be responsible for synovitis or joint trouble in turkeys; Snoeyenbos and Olesiuk[16] and Sevoian *et al.*[14] reported on a viral-like agent that caused turkey arthritis or synovitis.

It is the purpose of this paper to describe a case of tendon-sheath infection in turkeys from which a *Streptobacillus* was isolated and to present the experimental results of some transmission studies with this organism in turkeys and laboratory animals. To the best of our knowledge this is the first report of such infection in turkeys.

CASE HISTORY

During March 1957 a case was investigated in which approximately 10 percent of the breeder toms in one White Holland flock of about 100 toms showed lameness. Several days after this condition was noticed, the owner said that the toms became unable to stand, sat on their hocks and keels,

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and remained recumbent. They continued to eat and drink sparingly if food and water were within reach but lost weight and eventually died. They were housed in individual breeder stalls about 4 by 4 feet on several floors of a turkey-breeder barn. General management and sanitation appeared to be good except for a rat problem that could not be completely controlled by Warfarin or trapping. One large tom was brought back to the laboratory, observed for several days, and finally killed for examination. It would not stand unless prodded. It has lost some weight, but was not emaciated. The most notable gross lesions seen were swellings and puffiness in the area of the tendon sheaths immediately above and below both hocks. The posterior hock and sternal bursae were swollen and thickened possibly due to pressure from resting on them. The tissues overlying the hock joints were found to be thickened and edematous. There were no gross lesions of the bones or their articular surfaces. The tendon sheaths immediately above and below the hocks also were swollen and edematous. The lining of the sheaths was reddened. There was no free fluid, but a small amount of cloudy, yellowish-gray, mucoid and cheesy exudate was seen. The tendons did not appear to be affected. The liver was a dark reddish-bronze color with some greenish discoloration along the edges. It was firm on section. The bile was semisolid. The spleen was congested. The kidneys were slightly swollen, had a reddish-brown mottled appearance, and were full of urates. The air sacs were normal and the lungs showed only one very small area of congestion. The intestinal tract was empty and showed only a few slightly reddened areas of the mucous membrane in the midgut. Microscopic examination of intestinal scrapings showed a few trichomonads. A gram-negative pleomorphic *Streptobacillus* was isolated from the synovial exudate and the kidneys on blood agar, beef-heart-infusion-serum agar, beef-heart-serum broth, and by yolk-sac inoculation of 6-day-old chick embryos.

RESULTS

1. *Bacteriological studies.* Examination of the *Strep-*

tobacillus showed it to be a highly pleomorphic rod that varied in size from about 0.2 to 10 μ in length. In young cultures, single cells were common but with increasing age, filaments that contained granules and bands appeared. The presence of irregular bacillary, and coccobacillary elements gave the typical streptobacillary appearance (figure 1). After 48 hours of incubation in serum broth, large, spindle-shaped or spherical swellings were very common (figure 2). They occurred terminally, subterminally, and centrally in the rods and filaments. No branching could be detected.

The organism was not acid-fast. In very young cultures gram-positive forms were seen, but in older cultures all forms were gram-negative. It grew aerobically on blood-agar plates and in serum broth, but growth was enhanced under increased CO_2 tension. Its optimum temperature was about 37 C, but scanty growth occurred at 22 C.

In serum broth or in serum-thioglycollate medium the organism grew in whitish, small, granular spheres. In the former, the growth was confined to a granular sediment and the supernatant fluid remained clear. In the latter, growth was scanty or lacking in the upper 0.5-cm level of the medium. Below this mark it appeared as granular spheres suspended in an otherwise clear semiliquid with only an occasional formation of slight, general turbidity. It produced no odor and tests for the presence of indol and hydrogen sulfide were negative.

The organism showed no marked avidity in attacking sugars, but after 5 to 12 days of incubation arabinose, dulcitol, glucose, salicin, and sorbitol were fermented when exposed in serum broth. Lactose, maltose, mannitol, sucrose, and trehalose were not attacked. Methylene-blue milk was not reduced.

The microorgansim proved to be pathogenic for embryonated hens' eggs. Inoculation within the yolk sac produced death within 4 to 7 days and the organism was isolated readily from this site by seeding blood-agar plates rather heavily with inoculum. Growth could be seen after 36 to 48 hours of incubation. On heavily seeded plates, colonies ap-

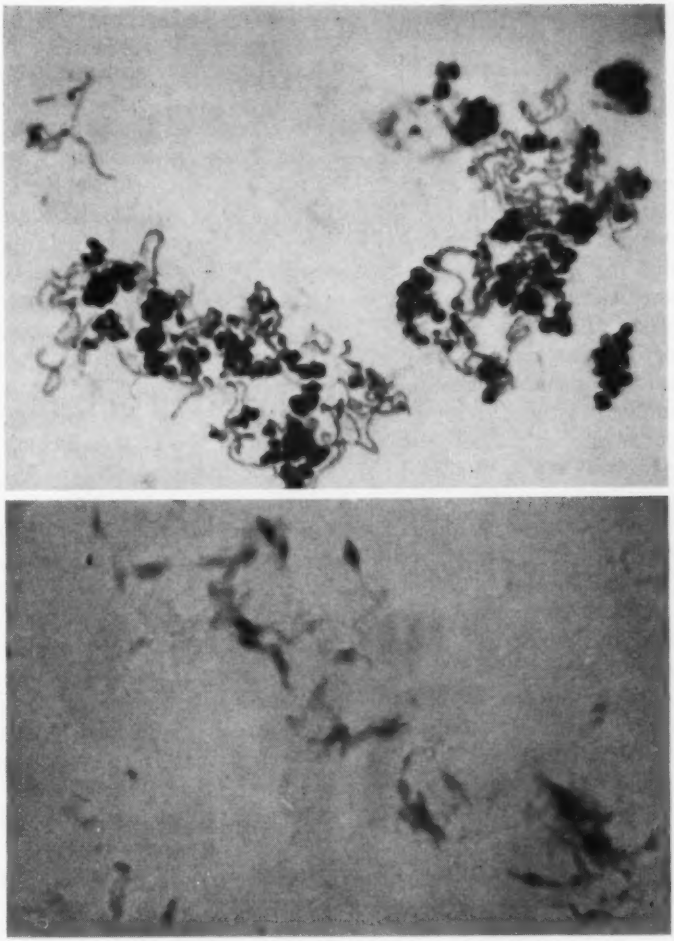

Fig. 1 (top). Gram-stain of a 48-hour-old blood agar culture of the organism showing the streptobacillary appearance. X 1,000.

Fig. 2 (bottom). Methylene blue stain of a 48-hour-old serum-broth culture showing the spindle-shaped swellings in the rods and filaments. X 1,000.

peared just about as readily under aerobic conditions as they did under increased CO_2 tension. Discrete colonies were 0.5 to 2 mm in diameter. They were circular, low-convex, and colorless with a smooth glistening surface. They did not hemolyze blood.

Filtration through a Seitz filter removed the organism. Such filtrates were free of bacteria upon culture and were nonpathogenic for eggs, whereas whole serum-broth cultures made after 15 transfers in serum broth or in thioglycollate medium remained pathogenic for chick embryos. Mortality in 5- to 9-day-old embryos inoculated via the yolk sac or chorioallantoic membrane was usually 100 percent. Practically all of the eggs died between the 2nd and 6th days postinoculation. The only gross lesions were congestion, occasional hemorrhages on the surface of the embryo, and stunting in those living 5 days or more. White opaque plaques 4 to 6 mm in diameter were produced on the chorioallantoic membrane when this route of inoculation was used.

The *Streptobacillus* was nonpathogenic for rabbits and hamsters. Cultures did not always kill mice upon intraperitoneal injection, but inoculation of yolk-sac material taken from infected embryos proved fatal within 2 to 3 days. The organism produced a peritonitis and was readily cultured from the peritoneal fluid and from the heart blood of the dead mice.

This culture, in nearly all respects, fits the description of *Streptobacillus moniliformis* as outlined in Bergey's Manual.[2] There appears to be no doubt but that it falls in the genus *Streptobacillus* and our studies indicate that *moniliformis* should be the species designation. Because we were dealing with only one culture and did not compare it directly with standard strains of *S. moniliformis* it is possible that future studies may prove it to be conspecific, a variant, or a turkey-adapted species.

2. *Poult transmission trials.* Negative results were obtained when poults 3 to 6 weeks of age were inoculated by intravenous, intramuscular, or foot-pad routes with whole

blood, liver, spleen, or kidney suspensions from the affected field case. When poults 3 to 11 weeks of age were inoculated by the intravenous or foot-pad routes with exudate from infected tendon sheaths, symptoms of pain, lesions of swelling and redness around the hock (figure 3), wing, and foot-pads, and mortality were produced. It made no difference whether the tendon-sheath exudate was from the original field case or from infected experimental poults. Broth or agar cultures of the *Streptobacillus* as well as yolk material from infected chick embryos also reproduced the disease in experimental poults.

Ninety-six poults were used in 11 transmission trials. Tendon-sheath exudate, both fresh or frozen for 8 months, produced lesions in poults but the frozen exudate was infectious only via foot-pad inoculation. Serum-broth cultures no older than 48 hours induced lesions. Lesions and mortality were produced most frequently in poults when either yolk from chick embryos infected with exudate or from embryos infected by serum-broth cultures were used as inoculum. Feeding of infective yolk failed to produce lesions in five poults. Intramuscular inoculation of six poults failed, but only three inoculated intravenously with proven infective material were negative. Out of 61 poults inoculated intravenously, 31 developed lesions around the hock, wing, or foot-pads and 15 died. Of 16 poults that were inoculated via the foot-pad, 12 developed lesions and two died.

3. *Chick transmission trials.* Six 4-week-old chicks were inoculated intravenously, three with proven infective yolk, and three with broth cultures. None developed symptoms or lesions within 30 days of observation.

DISCUSSION

Intravenous and foot-pad inoculations were the only routes that produced lesions in turkeys. Of these two, the foot-pad route had the higher percentage of takes but a lower mortality. Redness, swelling, and evidence of pain was seen in some poults by the end of the 2nd day postinoculation. Intravenous inoculations usually produced hock swellings

Fig. 3. The swollen hocks of an infected poult *(right)* are contrasted with those of a normal poult *(left)*.

first and sometimes wing and foot-pad swellings later. Foot-pad inoculations always produced swellings in the foot-pad first, followed occasionally by wing or hock lesions. When exudate or yolk material that had been frozen for 8 months was inoculated, only the foot-pad route produced swollen hock sheaths. The failure of this organism to induce infection in chickens by the intravenous route or to induce infection in turkeys by other than the intravenous or foot-pad routes distinguishes it from the avian infectious synovitis agent.

Human infections with a *Streptobacillus* were first reported by Levaditi, Nicolan, and Poincloux[11] in 1925. They named the organism *Streptobacillus moniliformis* because of its distinctive morphology. Since that time a number of cases in man of so-called "rat-bite fever" and a number of human cases with similar symptoms but unassociated with rat bites have been reported as caused by this microorganism or other bacteria very similar to it. The lack of a generally acceptable classification, however, has not only obscured much work on this subject but has led to numerous descriptions of this organism under different names. Brown and Nunemaker[3] in 1942 did much to clear up the confusion in the literature regarding the role of the *Streptobacillus* in human rat-bite fever. They included much important data on the morphology and cultural characteristics of this organism, and opposed Klieneberger's[10] theory that L forms of this organism should be classed as symbionts, and are pleuropneumonialike organisms. According to these authors, the principal animal reservoirs of *S. moniliformis* are mice and rats. Most cases in man have been traced to rat bites, but a few cases on record show no history of contact with this rodent. In fact, one of the most important epidemics occurred in Haverhill, Massachusetts, where milk that contained the organism was reported to be the source. The bacterium isolated from the blood and joint fluids of affected patients was called *Haverhill multiformis*,[13] but it is now believed that the organism actually was *S. moniliformis* or a very close associate.

To the best of our knowledge there are no reports in the

literature of a *Streptobacillus* causing infections in turkeys. With the exception of one other report by Theobald Smith[15] in 1918 of isolating the same or a similar type of organism from calves with pneumonia there seems to be no reports of *Streptobacillus* infections in animals other than in rats and mice and occasionally in an experimental rabbit.

One of the interesting things about this case is that in some of the human cases, arthritis, joint swelling, and pain are recorded in addition to fever. We did not study the temperature of the turkeys but the only visible lesions involved the joints or their tendon sheaths. In our field case, the owner complained of having trouble with rats in his barn. He said that he definitely saw evidence of rat bites on some of the breeder birds, especially around the vent, and thought they were bitten at night while on the lower roosts.

Heilman and Herrell[6] reported on the value of penicillin in the treatment of experimental infections with *S. moniliformis* in mice.

SUMMARY

The history, symptoms, and postmortem findings of a tendon-sheath infection in turkeys is described. Ten percent of the breeder toms in a barn had sore and swollen hocks. They became recumbent and eventually died.

A gram-negative *Streptobacillus* was isolated from the edematous and swollen tendon sheaths. Experimental poults became infected via intravenous and foot-pad routes of inoculation when tendon-sheath exudate, serum-broth cultures, or yolk from infected chick embryos was used as inoculum. The poults showed the same symptoms and postmortem lesions as the field case. Ninety-five to 100 percent of 5- to 9-day-old chick embryos were killed when inoculated with infective material via the yolk sac or chorioallantoic membrane. Chickens were not infected by intravenous inoculation of infective exudate or cultures of the *Streptobacillus*. Mice were infected, but hamsters and rabbits proved to be refractive. The organism was identified tentatively as *Streptobacillus moniliformis*. It could possibly be a variant or turkey-

adapted strain of this organism.

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FURAZOLIDONE MEDICATION IN CHICKENS EXPERIMENTALLY INFECTED WITH *SALMONELLA PULLORUM**

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IN A previous publication, Smyser and Van Roekel[5] reported on the influence of furazolidone medication upon chickens of various ages experimentally and naturally infected with *Salmonella pullorum*. The drug failed to reduce the agglutination titers of naturally infected adult birds and the organism was recovered from 75 percent of the birds. In experimentally infected chickens the higher drug levels administered were more effective in preventing the establishment of the infection. The drug treatment administered during the early stages of the disease appeared to be more effective than when the medication was initiated several days post-infection. The findings of other investigators were reviewed previously.

This report presents the results of continued investigations with experimentally infected birds.

MATERIALS AND METHODS

The chickens used in these investigations originated from pullorum-clean sources. The White Leghorn (WL) cockerels, used in trials 1 and 2, were obtained from laboratory hatched eggs and the Rhode Island Red (RIR) hens, used in trials

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3, 4, and 5, were received from the University Experimental Farm. Preinfection blood samples were collected in all trials to reaffirm the pullorum-free status. The pullorum culture was the same as used previously but which had been reisolated from the birds in other experiments. The birds were infected *per os* with a 24-hour broth culture using a glass syringe fitted with a blunt needle.

Table I presents the experimental design for the various trials. The birds in trials 1, 3, and 5 were paired in cages, and in trials 2 and 4, the birds were maintained in colony houses. Furazolidone* medication of the caged birds was by tablets and for the housed birds it was mixed in the feed.

In trials 1 and 3, the chickens in groups 1 and 1a were medicated for 10 days beginning at time of initial infection; groups 2 and 2a were medicated for 10 days beginning one week after initial infection. In trial 5, the birds in group 1 received furazolidone tablets for 14 days and those in group 2 for 28 days, beginning at time of initial infection. The control birds in trials 1, 3, and 5 were infected but not medicated.

All the chickens in trial 2 received the medicated feed for 21 days beginning at time of infection, but only half of them were infected with *S. pullorum*. Trial 4 was conducted in two houses with half of the birds in each house being infected. The birds in one house received medicated feed for 2 weeks beginning at time of infection and those in the second house were left unmedicated.

All birds were bled weekly and the sera were tested by the macroscopic tube agglutination method in two-fold serial dilutions beginning with the 1:10 dilution. The tests were read after 24 hours incubation at 37 C and at room temperature overnight. In all trials either a 2+ reading in the 1:20 dilution or a 1+ reading in the 1:40 dilution was considered as the minimum criterion for a positive titer. The cultures made at necropsy and the procedure for bacteriological examination were the same as previously reported.[5]

* The furazolidone was obtained through the courtesy of Hess and Clark, Inc., Ashland, Ohio.

TABLE I

Design of studies of the effect of furazolidone upon artificially pullorum-infected chickens

Trial	Test group	Number of birds	Age	Sex	S. pullorum inoculum*	Furazolidone medication daily dosage	Duration of trial
1	1	6	9 weeks	M	2 doses of 1.0 ml 5 days apart	20 mg (0.027%)	54 days
	1a	6				40 mg (0.055%)	
	2	6				20 mg	
	2a	6				40 mg	
	Control	8				—	
2	1	25	14 weeks	M	2 doses of 1.0 ml 4 days apart	0.044% level in feed	37 days
	2	25			—	0.044% level in feed	
3	1	6	2 years	F	3 daily doses of 1.0 ml	30 mg (0.022%)	63 days
	1a	6				50 mg (0.037%)	
	2	6				30 mg	
	2a	6				50 mg	
	Control	7				—	
4	1	12	2 years	F	3 daily doses 1.0 ml	0.011% level in feed	56 days
	2	13			—	0.011% level in feed	
	3	13			3 daily doses 1.0 ml	—	
	4	11			—	—	
5	1	8	1 year	F	3 daily doses of 1.0 ml	15 mg (0.011%)	56 days
	2	8				15 mg	
	Control	7				—	

* Dosage varied from 3×10^7 to 1.5×10^8 viable organisms per ml.

RESULTS

Trial 1. The cockerels in groups 1 and 1a which received medication for 10 days beginning at time of infection remained essentially negative to the tube agglutination test throughout the trial. In groups 2 and 2a where medication did not start until one week after initial infection, all 12 birds were positive by the second week and showed the following distribution of titers: one bird with 80, three with 160, and eight with 320. The titers declined steadily and were negative at the end of the trial.

By the second week after infection all eight of the controls were positive. Seven birds showed a titer of 320 and one bird a titer of 80. A gradual decline followed until the fifth week when the titers leveled off. At the end of the trial five of the eight controls were still positive.

None of the birds in this trial revealed any gross pathologic lesions at necropsy, and *S. pullorum* was not isolated.

Trial 2. None of the birds in this trial developed a serological response to the inoculated organism. There were no gross lesions at necropsy and the organism was not isolated.

A summary of the serologic, bacteriologic, and the gross pathologic results in trials 3, 4, and 5 is presented in table II.

Trial 3. The birds were medicated with furazolidone tablets at 30 mg and 50 mg per bird. These dosage levels approximated 0.022 percent and 0.037 percent of the drug in the feed.

Three of the six birds in group 1 were serologically positive (titers of 80, 320, 320) by the third week after initial infection and remained so for the duration of the experiment. Five birds exhibited gross pathologic lesions at necropsy and *S. pullorum* was isolated from the three serologically positive birds.

All the birds in group 1a were essentially negative to the tube dilution test throughout the trial. One bird showed gross pathologic lesions but the organism was not isolated.

TABLE II

Results of studies of furazolidone medication in experimental pullorum-infected hens

Trial	Test group	Number of birds	Number of birds with gross pathology					Total number of birds positive		
			Peri-cardial	Hepatic	Splenic	Peri-toneal	Ovar-ian	Lesions	Sero-logical	Bacterio-logical
3	1	6	0	3	0	4	3	5	3	3
	1a	6	1	0	0	0	0	1	0	0
	2	6	0	2	0	2	3	3	3	2
	2a	6	1	1	1	1	0	2	1	0
	Control	7	4	3	0	5	3	7	7	7
4	1	12	3	6	0	3	4	8	10	7
	2	13	0	3	0	0	2	5	1	2
	3	13	0	5	0	5	2	9	10	11
	4	11	1	3	0	0	0	3	1	1
5	1	8	1	1	1	0	2	2	2	2
	2	8	1	0	0	1	2	2	2	2
	Control	7	3	0	0	2	4	7	7	7

By the first week after infection, five of the six birds in group 2 were serologically positive and three were still positive at the end of the trial with titers of 80, 640, and 640. These three birds revealed gross pathologic lesions at necropsy and *S. pullorum* was isolated from two of them.

All six birds in group 2a were serologically positive by the second week, but only one was positive at the end of the trial with a titer of 80. There were no isolations of the organism at necropsy although two birds revealed slight gross pathologic lesions.

Five of the seven control birds were positive to the tube agglutination test by the first week and all were positive by the third week. The titers at necropsy ranged from 80 to 640. At necropsy all seven birds exhibited gross pathologic lesions and yielded *S. pullorum*.

The mean serological titers by weeks are presented graphically in figure 1. These graphs represent all the birds within a group and not just the ones which are serologically positive. When the birds were medicated at time of infection, the 30 mg daily dosage (0.022 percent) was effective only for the two weeks the birds received furazolidone after which the titer rose sharply. On the other hand, the 50 mg daily dosage (0.037 percent) prevented a serological response throughout the entire trial.

When the furazolidone medication was started one week after initial infection, the 30 mg daily dosage did not cause a drop in serological titer. Again the 50 mg daily dosage appeared to be more effective in that a steady decline occurred.

Trial 4. In this trial the birds in one house were on medicated feed at the 0.011 percent level, the second house was not medicated, and only half the birds in each house were infected with *S. pullorum*.

Eleven of the 12 infected, medicated birds were serologically positive by the second week after infection and 10 were still positive at the end of the trial with a titer range of 40 to 1280. Gross pathologic lesions were exhibited by eight birds and seven yielded *S. pullorum*.

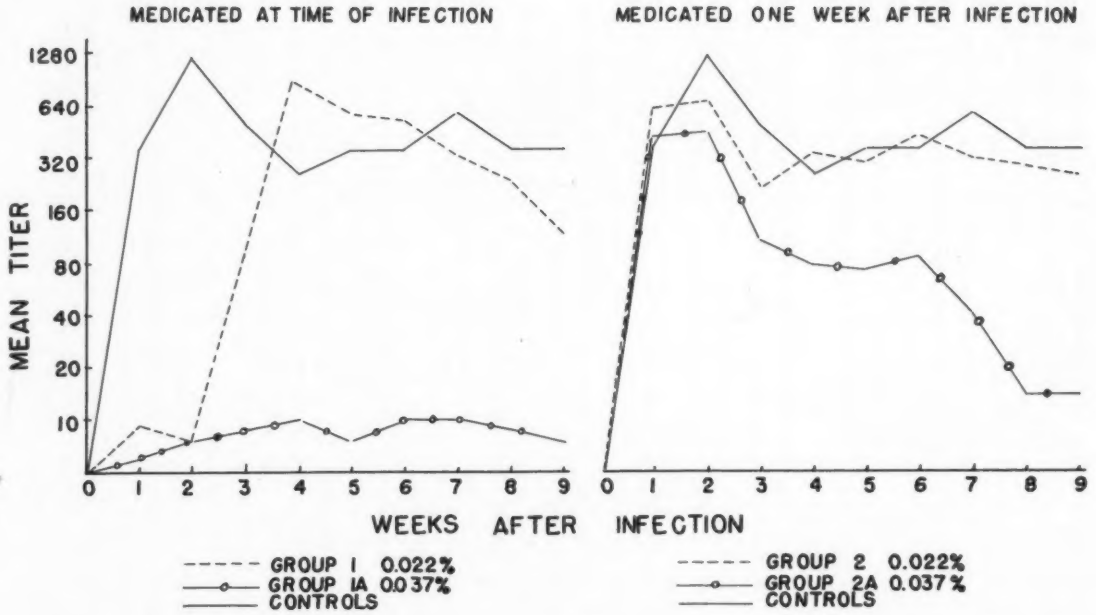

Fig. 1. Influence of furazolidone medication upon serological titers of hens experimentally infected with *S. pullorum*—Trial 3.

In the non-infected, medicated group one bird at the fifth week had developed a slight titer which increased to 1:2560 at the end of the trial. Gross pathologic changes were noted and the organism was isolated. Another bird yielded *S. pullorum* from a sternal abscess although there were no other lesions and no serological titer. Three other birds in this group revealed slight gross pathologic lesions.

All of the 13 infected, non-medicated birds were serologically positive by the second week, but only 10 were positive when the trial terminated with a titer range of 160 to 2560. Nine birds exhibited gross pathologic lesions at necropsy and 11 yielded *S. pullorum*.

In the non-infected, non-medicated group, one bird became serologically positive at the fourth week and had a titer of 1280 at the end of the trial. Gross pathologic lesions were revealed and the organism was isolated. Two other birds in this group showed slight gross lesions.

The graphical representation of the weekly mean serological titers is presented in figure 2. The mean titers represent the entire group although only one bird in each of the non-infected groups was positive. Furazolidone medication caused a slight delay in the development of serological titers in the experimentally infected birds. By the fourth week after infection there was little difference between the medicated and the non-medicated groups of infected birds.

Trial 5. Eight birds were medicated at time of infection with furazolidone tablets at 15 mg per bird for 14 days and a second group of eight birds for 28 days. This dosage approximated the 0.011 percent level of the drug in the feed.

Two of the eight birds medicated for 14 days were serologically positive (titers of 160 and 320) at the fourth week. One of the positive birds died during the seventh week. At necropsy, both birds revealed gross pathologic lesions and both yielded *S. pullorum*.

One of the eight birds medicated for 28 days developed a strong titer (320) at the sixth week, or two weeks after medication ceased, and a second bird developed a titer of 80 at the eighth week. Gross pathologic lesions were exhibited

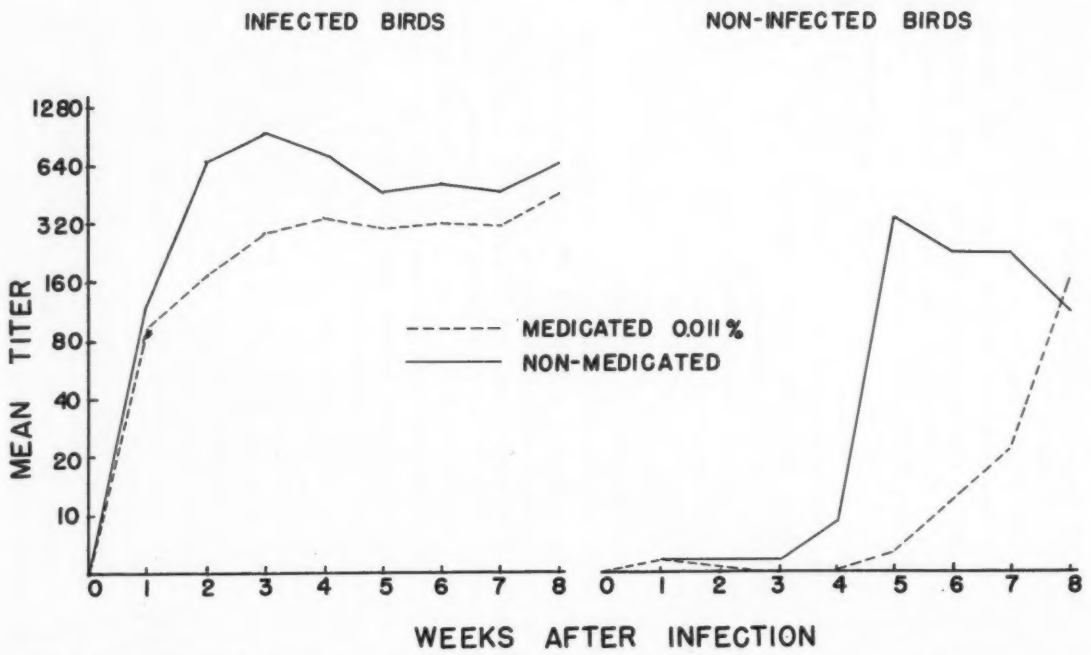

Fig. 2. Influence of furazolidone medication upon serological titers of hens experimentally infected with *S. pullorum*—Trial 4.

by these birds and *S. pullorum* was recovered.

All seven of the control birds were strongly positive by the second week after infection with a titer range of 160 to 1280 and remained so for the duration of the trial. At necropsy, all seven revealed gross pathologic lesions and yielded the organism.

The weekly mean serological titers are presented graphically in figure 3. The mean titers represent all the birds in a group, although only two birds were positive in each of groups 1 and 2. It will be noted that the positive titers in the medicated birds had developed by the second week after medication had ceased, except for one bird in group 2 which was not positive until the fourth week after medication ceased.

DISCUSSION

The results of the two trials with WL cockerels differed from the results previously obtained with young RIR cockerels. Infection, as demonstrated by serological titer, was established only in the controls and the birds medicated one week after infection in trial 1, and in none of the birds in trial 2. In trial 1, the birds medicated one week after infection (groups 2 and 2a) were serologically positive when treatment started and were negative at the end of the trial. This would suggest that medication was effective in controlling the infection. However, three of the control birds which had been strongly positive at the second week, were negative at 8 weeks when the trial terminated. This inability to establish pullorum infection may be due in part to the more resistant nature of cockerels, especially of the WL breed. Resistance of WL birds to pullorum disease and fowl typhoid has been observed by other workers.[1,3,4]

Although five of the cockerels in trial 1 were serologically positive at the end of the trial (titers of 160, 40, 320, 40, 40), *S. pullorum* was not isolated even from the birds with the higher titers. Titkemeyer and Schmittle[6] have reported on the difficulty of isolating *S. gallinarum* from WL inoculated chicks after furazolidone medication. They made no attempt to determine whether this was a temporary in-

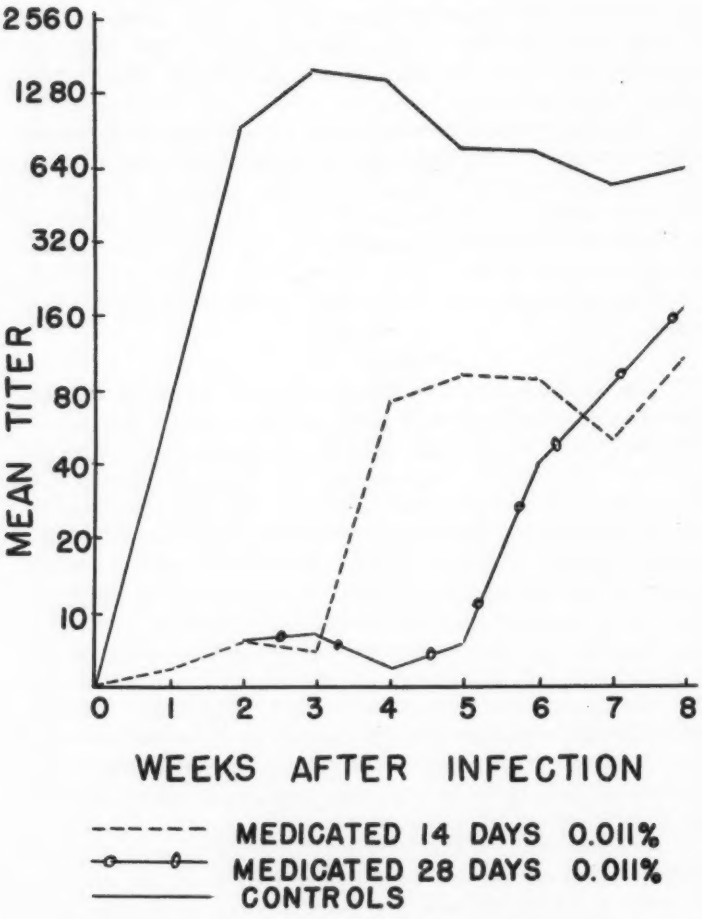

Fig. 3. Influence of furazolidone medication upon serological titers of hens experimentally infected with *S. pullorum*—Trial 5.

hibition or a complete elimination of the organism.

In trial 3, the higher level of furazolidone (0.037 percent) appeared more effective than the lower level (0.022 percent) in preventing and controlling experimental pullorum infection in hens. The birds medicated at the higher level at time of infection did not develop serological titers after medication ceased, whereas three of the six birds at the lower level developed positive titers the second week after medication ceased. Where the infection had developed before medication started, the higher level was more effective in controlling the disease as evidenced by a decline in serological titers.

In trial 4, hens received medicated feed at the 0.011 percent level for 14 days. Although the medication caused a slight delay in the development of a serological response, it did not prevent the development of good serological titers after the medicated feed was withdrawn. Force-medication in trial 5 at the 0.011 percent level resulted in a delay in the serological response. When furazolidone was withdrawn, two birds medicated for 14 days and two birds medicated for 28 days developed strong titers and yielded the organism at necropsy. Henderson *et al.*,[2] have reported that the 0.011 percent level in the feed did not materially affect the serological titers of experimentally infected adult birds.

An interesting observation in trial 4 was the relative lack of cross infection between infected and non-infected birds. In each house only one-half the birds were infected, and although they mingled freely, only one of the non-infected birds in each house developed a serological titer.

With adult chickens, Henderson *et al.*,[2] also observed that furazolidone medication did not affect the recoverability of the organism, but that the ability to isolate *S. pullorum* depended more upon the final agglutination titers. Similar findings were observed in this and in previous investigations.[5] Primarily, birds with low positive titers failed to yield *S. pullorum*. Serological titers may decline several weeks after furazolidone medication has ceased. Those birds, which do not become serologically positive until after treatment

has stopped, do not usually show a decline in titer.

There was a difference between trials 4 and 5 in the number of birds developing a titer after medication stopped. In trial 4, the birds were in colony houses with free access to a screened porch and received medicated feed. The birds in trial 5 were paired in cages and were force-medicated, thereby receiving a definite amount of furazolidone each day. Although no visible symptoms were noted, the birds in trial 4 may not have consumed enough of the medicated feed in the early stages of the disease to overcome the organism. This may explain partly the difference in the number of medicated birds developing titers.

In a natural outbreak of pullorum infection among pullets and mature birds, furazolidone medication would not begin until the disease had become established in at least part of the flock. Thus, the advantages of early medication may not be available. The purpose of medication is to reduce the severity of the disease in those birds already infected and to prevent further spread of the infection. The effectiveness of furazolidone at different levels and for varying periods of medication must be more thoroughly explored for infected birds under field conditions.

SUMMARY

The effectiveness of furazolidone medication was investigated in chickens artificially infected with *S. pullorum*.

In two groups of young White Leghorn cockerels, the influence of furazolidone could not be ascertained because of difficulty in establishing the infection.

In hens force-medicated at two different levels, the 50 mg daily dosage (0.037 percent) was more effective than the 30 mg daily dosage (0.022 percent) in reducing the incidence of infection when medication started at time of infection and one week after infection and in both cases continued for 10 days.

There was little difference in the incidence of infection between medicated and non-medicated infected hens which received the 0.011 percent level of furazolidone in the feed for 14 days.

Force-medication at the 15 mg daily dosage (0.011 percent) for 14 and 28 days after infection did not prevent some of the hens from developing serological titers after the medication ceased.

The influence of furazolidone appears to depend upon the initiation, duration, and level of medication. More information is needed for the proper application of this relationship in an eradication program.

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IN VITRO RESISTANCE OF AVIAN PPLO TO ANTIBACTERIAL AGENTS* †

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IN VITRO tests of the effectiveness of antibacterial agents on avian pleuropneumonia-like organisms (PPLO) have been thoroughly investigated by several workers. A recent review of this subject[3] shows that many workers have found most of the commonly used antibacterial agents to exert a suppressing effect on PPLO. In general, this information was obtained by exposing constant numbers of PPLO to graded amounts of antibacterial agents.

When natural PPLO infections are treated, an unknown but variable number of organisms is subjected to the action of known amounts of antibacterial agents. It would seem desirable to attempt, in so far as possible, to duplicate these conditions by determining the effects of graded concentrations of antibacterial agents on graded numbers of PPLO. The purpose of this paper is to report the results of such a study.

MATERIALS AND METHODS

The Winchester (W) strain of PPLO was used as the test organism in this study. This organism was chosen because it is regarded as a typical pathogenic PPLO and it has been carried *in ovo* without exposure to antibiotics in this laboratory since its isolation from chickens undergoing

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a typical outbreak of chronic respiratory disease in 1950. The culture used for this study had been passed in media[s] for from 20 through 39 passages.

The antibacterial agents tested in this experiment were furazolidone, tetracycline, streptomycin and chloromycetin. These agents were prepared in duplicate for testing by separately adding either no antibacterial agent, 0.1, 1.0, 10, 100, 1,000, or 10,000 microgram/ml amounts of the agents to sets of media.[a] Each of the seven sets of media thus prepared were inoculated with 10 percent inocula. The inocula were prepared by making 10 fold dilutions, ranging from undiluted through 10^{-10}, of a 3 day broth culture of PPLO. These cultures were incubated at 37 C for 45 days and read twice weekly for presence or absence of growth throughout that period of time. The presence of growth was determined by visible turbidity.

A second set of tubes, prepared as above but inoculated, was incubated at 37 C for two weeks prior to being identically inoculated. After inoculation these cultures were read for growth following the same time schedule used for the first set of cultures.

In an additional identical experiment using only streptomycin, the first cultures to grow in each concentration were tested for their ability to grow in all concentrations of that antibiotic.

RESULTS

Streptomycin (*table 1*). Streptomycin did not suppress but did slightly delay the growth of PPLO at concentrations of 0.1 and 1.0 microgram/ml. In concentrations of 10, 100 and 1,000 micrograms/ml of the antibiotic, growth was greatly retarded and suppressed. No growth occurred at the 10,000 microgram/ml concentration.

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[a] Difco PPLO broth without crystal violet, 1% Difco serum, 1/4000 parts thallium acetate, adjusted to a final pH of 7.8.

[s] Difco PPLO broth without crystal violet, 1% Difco PPLO serum fraction, 1/4000 parts thallium acetate, 1,000 units penicillin per ml, final pH adjusted to 7.8.

TABLE I

*In vitro** PPLO resistance to streptomycin

Days post inoculation	Micrograms/ml. Streptomycin**											
	0		0.1		1.0		10		100		1000	
	1st trial	2nd† trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
3	3‡	5	3	5	2	3		1				
7	6	5	6	5	4	3	1	1				
10	7	7	8	7	7	7	1	4	1	1		
14	7	7	8	7	7	7	1	4	1	1		
17	7	7	8	7	7	7	1	4	1	1		
21	7	7	8	7	7	7	1	4	1	1		
24	7	7	8	7	7	7	1	4	1	1	1	
45	7	7	8	7	7	7	1	4	1	1	1	

* Difco PPLO broth with 1% serum fraction and 1/4000 parts thallium acetate adjusted to PH 7.8 after the addition of streptomycin.

** A 10,000 microgram level was also run.

† The second trial was identical to the first except that the complete test medium was incubated at 37 C for 2 weeks prior to inoculation.

‡ Values represent the greatest inoculum dilution (expressed as the reciprocal of the log) which initiated growth.

In the first trial of this experiment, viable PPLO were obtained from tubes containing no antibiotic, and 10 micrograms/ml of antibiotic, 53 and 57 days respectively, after initial growth appeared. Viable PPLO were not detected at this time in the tubes containing 0.1, 1.0, 100, or 1,000 microgram/ml concentrations of streptomycin.

In the second trial of this experiment, viable PPLO were obtained 28 days after the initial appearance of growth, from the tubes containing no antibiotic and from tubes containing all except the 10,000 microgram/ml concentration of antibiotic.

In an identical experiment, subcultures from the first cultures to grow in streptomycin grew in all concentrations of the antibiotic within four days after subculture. Twenty-five percent inocula were used when these subcultures for viable PPLO were made.

Chloromycetin (*table 2*). The growth of PPLO was not suppressed but was somewhat retarded by the 0.1 microgram/ml concentration of chloromycetin. The 1.0 microgram/ml level retarded and suppressed growth. In the first trial no growth occurred in the 10 microgram/ml concen-

tration, and it was both retarded and suppressed in the second trial by this concentration of chloromycetin. No growth occurred in 100, 1,000 or 10,000 microgram/ml concentrations of the antibiotic.

Tetracycline (*table 3*). In the first trial of this experiment, growth was slightly suppressed by 0.1 microgram/ml of tetracycline. It was both retarded and suppressed by 1.0 microgram/ml of the antibiotic. Growth did not occur in 10, 100, 1,000, or 10,000 microgram/ml of this substance.

In the second trial growth occurred at a low level in tubes containing no antibiotic and in those containing 0.1, 1.0 and 10 micrograms of tetracycline per ml.

Furazolidone (*table 4*). In the first trial of this experiment, growth was retarded by 0.1 and 1.0 micrograms/ml

TABLE II

In vitro* PPLO resistance to chloromycetin

	Micrograms/ml Chloromycetin**									
	0		0.1		1.0		10		100	
Days post inoculation	1st trial	2nd† trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
3	3‡	2	2	3	2	2				
7	5	5	4	5	4	2		1		
10	6	5	6	5	4	4		1		
14	6	5	7	5	4	4		1		
17	6	8	7	7	4	4		1		
21	6	8	7	7	4	4		1		
24	6	8	7	7	4	4		1		
28	6	8	7	8	5	4		1		
45	6	8	7	8	5	4		1		

* Difco PPLO broth with 1% serum fraction and 1/4000 parts thallium acetate adjusted to pH 7.8 after the addition of chloromycetin.

** 1,000 and 10,000 microgram levels were also run.

† The second trial was identical to the first except that the complete test medium was incubated at 37 C for 2 weeks prior to inoculation.

‡ Values represent the greatest inoculum dilution (expressed as the reciprocal of the log) which initiated growth.

of furazolidone, and did not occur in 10, 100, 1,000 or 10,000 microgram/ml concentrations of the drug. In the second trial growth was suppressed by 0.1 and 1.0 microgram/ml concentrations of the drug, suppressed and retarded by the 10 microgram concentration and did not occur in the presence of 100, 1,000 or 10,000 microgram/ml concentrations.

TABLE III

In vitro* PPLO resistance to tetracycline

Days post inoculation	Micrograms/ml Tetracycline**									
	0		0.1		1.0		10		100	
	1st trial	2nd† trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
3	2‡	3	2	3	1	3		3		
7	5	3	4	3	1	3		3		
10	8	3	7	3	1	3		3		
14	8	3	7	3	3	3		3		
17	8	3	7	3	4	3		3		
45	8	3	7	3	4	3		3		

* Difco PPLO broth with 1% serum fraction and 1/4000 parts thallium acetate adjusted to pH 7.8 after the addition of tetracycline hydrochloride.

** 1,000 and 10,000 microgram levels were also run.

† The second trial was identical to the first except that the complete test medium was incubated at 37 C for 2 weeks prior to inoculation.

‡ Values represent the greatest inoculum dilution (expressed as the reciprocal of the log) which initiated growth.

TABLE IV

In vitro* PPLO resistance to furazolidone

Days post Inoculation	Micrograms/ml Furazolidone**									
	0		0.1		1.0		10		100	
	1st trial	2nd† trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
3	3‡	3	2	3	2	1				
7	5	4	4	4	3	3				
10	6	8	6	7	4	7		1		
14	6	8	6	7	4	7		2		
17	6	8	6	7	4	7		2		
21	6	8	6	7	5	7		2		
24	6	8	6	7	6	7		2		
45	6	8	6	7	6	7		2		

* Difco PPLO broth with 1% serum fraction and 1/4000 parts thallium acetate adjusted to pH 7.8 after the addition of furazolidone.

** 1,000 and 10,000 microgram levels were also run.

† The second trial was identical to the first except that the complete test medium was incubated at 37 C for 2 weeks prior to inoculation.

‡ Values represent the greatest inoculum dilution (expressed as the reciprocal of the log) which initiated growth.

A comparison of the greatest degrees of growth suppression of PPLO in the presence of the four antibacterial agents (figure 1) shows that chloromycetin and tetracycline produce similar rates of suppression, and exert their initial

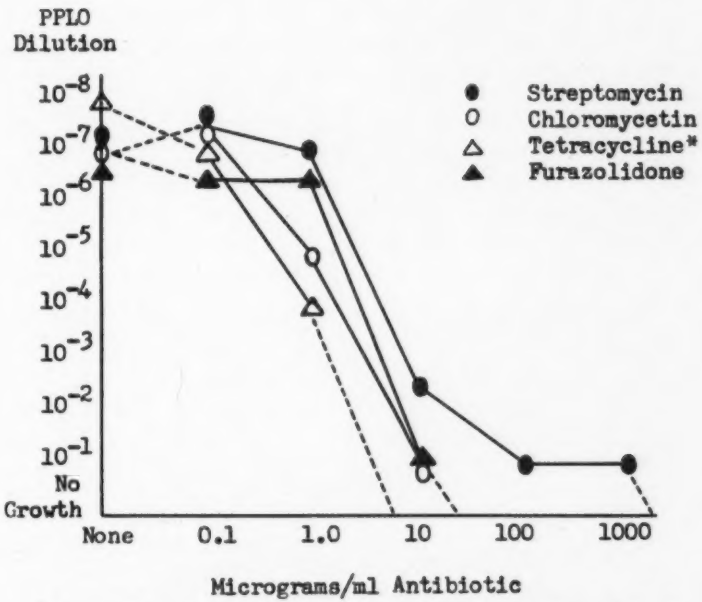

Fig. 1. The effect of various concentrations of antibacterial substances on graded PPLO populations. The results were obtained from two experiments. The complete medium used in the second experiment was incubated 2 weeks longer than that used in the first.
* Second trial excluded because of a laboratory error.

effect at a lower concentration than either streptomycin or furazolidone. Streptomycin and furazolidone produce little and no suppression at lower levels respectively, and about the same degree of suppression from the 1.0 to the 10 microgram/ml concentration levels. After this point, growth is completely suppressed by furazolidone; whereas, it continues to be suppressed to the same degree through the 100 and 1,000 microgram/ml concentrations of streptomycin, after which it is completely suppressed.

DISCUSSION

The data obtained from this experiment indicate that suppression of PPLO growth is approximately directly re-

lated to chloromycetin and tetracycline concentrations, but not to furazolidone or streptomycin concentrations. The strain of PPLO used in this study seems to exhibit the one step resistance to streptomycin which is characteristic of bacteria.[1] This information would seem to indicate that these antibacterial agents, particularly streptomycin, should be used at high levels to produce maximum therepeutic response when treating PPLO infections. In view of this, it appears that this type of study should be made with other antibacterial agents which are potentially useful in treating PPLO infections.

The observations that PPLO remain viable in media which have been incubated for as long as from 28 to 57 days, and that lag phases in the growth of PPLO last from 17 to 28 days (tables 1, 2, 3, and 4), suggest that the "killing"[2] and "lethal"[4] concentrations of antibiotic which have been reported might have been greater upon continued incubation.

SUMMARY

Graded concentrations, 0.1, 1.0, 10, 100, 1,000 and 10,000 micrograms/ml of furazolidone, streptomycin, chloromycetin and tetracycline were tested for their ability to suppress the growth of graded populations of the Winchester strain of PPLO.

PPLO growth was suppressed in approximately a direct relationship to inoculum concentration, and at similar rates by chloromycetin and tetracycline. Streptomycin and furazolidone exerted similar effects after an initial lag. After the rapid initial suppression of growth produced by streptomycin, a constant but reduced level of growth was initiated throughout the higher concentrations of streptomycin to the point where no growth was observed. Subculture of the PPLO growing in these concentrations of streptomycin suggests that this strain of PPLO may exhibit a one step resistance to streptomycin.

Under the conditions of the experiment, PPLO were observed to have a lag growth phase lasting as long as 28 days. It was possible to subculture the organism after it had been incubated for as long as 57 days.

ACKNOWLEDGMENTS

The author wishes to acknowledge the technical assistance of Barbara Ann Browning M.T./A.S.C.P.

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CONTROL OF INFECTIOUS SYNOVITIS. XI. THE POTENTIATING EFFECT OF TEREPHTHALIC ACID ON CHLORTETRACYCLINE*†

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CHLORTETRACYCLINE (CTC) has been used effectively in the control of infectious synovitis. However, the desired concentration (100 grams per ton) in the feed is expensive. Generally, 50 grams per ton has been satisfactory, but the presence of other clinical or sub-clinical infections often causes additional stress conditions such that 100 grams has proven necessary for clinical control.

Any practical technique which would enhance the utilization of the antibiotic by the bird should effect a corresponding reduction in the dietary quantity necessary for clinical control. Eisner *et al.*[1] reported that the serum concentration of chlortetracycline could be increased by simultaneous administration of dihydrogen phosphate or certain organic acids such as citric, malic or tartaric acids. Peterson[3] using chicks infected with *E. tenella*, reported that the incorporation of the organic compound, terephthalic acid (TPA), into a ration containing chlortetracycline potentiated its antibiotic activity and increased the blood serum concentration of chlortetracycline by a minimum of four times.

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This is a report of an *in vivo* study with infectious synovitis agent 1853 in chicks to determine the influence of terephthalic acid upon the biological activity of chlortetracycline HC1.

MATERIALS AND METHODS

Fifteen New Hampshire chicks were used in each treatment. Except for the non-infected, control treatments, the chicks in all treatments were inoculated via foot pad when 2 weeks old with infectious synovitis agent 1853. Additives to the basal ration were included when the chicks were a week old and were given continuously. The chicks were maintained in electrically heated batteries equipped with wire floors and were located in a constant temperature-humidity controlled room.

The basal ration was a commercial open formula broiler ration containing 3 grams of penicillin per ton. The design of the experiment is shown in table 1. Terephthalic acid (technical grade) was added to the basal ration at the rate of 0, 0.25, 0.5 or 1.0 percent. Chlortetracycline was added at the rate of 0, 12.5, 25, 50, 100 or 200 grams per ton of feed. In addition to this factorial design, a battery of non-inoculated chicks was maintained for each concentration of TPA.

The birds were observed and the mortality recorded daily. The severity of infection was determined and recorded weekly during the 4-week experimental period.

RESULTS

A comprehensive expression of the severity of the infectious synovitis in birds given the various treatments was determined by the number of infected birds, the degree of swelling as observed in the inoculated leg, the number of birds in which the infection had extended to joints other than those in the inoculated leg, mortality and weight gain. Results of these factors are summarized in table 1.

No signs of infectious synovitis appeared in the non-inoculated birds. When no CTC was in the ration, 59 of the 60 inoculated birds became infected even though 45 of these birds were receiving feed containing from 0.25 to 1.0 percent TPA. Thus, TPA *per se* at the above rates did not alter the in-

TABLE I

Effect of increasing concentrations of terephthalic acid and chlorotetracycline on the severity of infectious synovitis in experimentally inoculated chicks.*

Chlortetracycline HCl gm/ ton of feed	Percent of terephthalic acid in the feed																			
	0	0.25	0.5	1.0	0	0.25	0.5	1.0	0	0.25	0.5	1.0	0	0.25	0.5	1.0	0	0.25	0.5	1.0
	No. birds infected				Deg. of swelling				Extension				Mortality				Weight gain, gm.			
Not inoculated																				
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	484	499	545	542
Inoculated																				
0	15	14	15	15	8.8	8.8	8.8	8.8	14	14	15	15	8	5	8	8	69	107	71	85
12.5	15	12	8	1	8.3	2.7	1.9	.1	14	1	2	0	1	0	0	0	271	473	503	503
25	11	3	0	0	4.0	.3	0	0	5	0	0	0	0	0	0	0	425	535	513	536
50	7	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	515	502	531	517
100	1	0	0	0	.1	0	0	0	0	0	0	0	0	0	0	0	509	504	483	549
200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	518	551	546	565

* Severity of infection shown by the following: *number* of birds developing disease, *degree* of swelling—based on a scoring system of 0 to 9, applies only to leg of inoculation, *extension*—number of birds in which infection has extended to joints other than those in the inoculated leg.

fectivity of the infectious synovitis agent for chicks. With 12.5 grams of CTC per ton in the ration, the number of birds which became infected was reduced from 15 to 1 as the concentration of TPA was increased from 0 to 1.0 percent. With 25 grams of CTC, 11 birds were infected if no TPA was included in the ration but only three if 0.25 percent TPA was present and none if higher rates of TPA were provided. Seven birds at 50 grams and one bird at 100 grams of CTC were infected when no TPA was present in the feed. Addition of 0.25 to 1.0 percent TPA to these feeds inhibited the disease and no signs of infection were observed. None of the birds became infected if they were consuming a ration with 200 grams CTC per ton.

The average degree of swelling was obtained by scoring from zero to nine the swelling of the inoculated leg and was observed to be the same for the infected birds when no CTC was provided. Terephthalic acid *per se* did not reduce the magnitude of the swelling when CTC was not given. Birds given 12.5 grams of CTC showed a slight though insignificant reduction in the average degree of swelling. However, as the concentration of TPA was increased from 0 to 1.0 percent, the average degree of swelling was reduced from 8.3 to 0.1. With 25 grams of CTC, an average swelling of 4.0 and 0.3 was recorded for the chicks receiving no TPA and 0.25 percent TPA, respectively. When higher levels of TPA were used no swelling was apparent. Although an average swelling of 1.0 and 0.1 occurred at 50 and 100 grams of CTC, respectively, none was observed if TPA was also present in the ration at the indicated amounts.

Extension of the infection in the birds occurred in essentially all of the infected controls. When 12.5 grams of CTC was in the ration, 14 of the 15 birds showed the generalized systemic infection. With the addition of 0.25, 0.5 or 1.0 percent of TPA, only 1, 2 and 0 birds, respectively, were observed to have this generalized infection. Twenty-five grams of CTC without TPA was effective to restrict extension of the infection so that only five birds were so affected. With the addition of TPA no spread was detectable. At 50 grams

of CTC or higher, with or without TPA, no evidence indicating a generalized infection was apparent during the experiment.

Although a slight increase in weight gains occurred in the non-infected control birds as the concentration of TPA increased to 0.5 percent, additional studies are necessary to test its significance. Weight gains of the infected controls were substantially lower than the non-infected control treatments. The severity of the infection in the birds that got only 12.5 and 25 grams of CTC was great enough to suppress weight gains. Otherwise, weight gains were similar to the non-infected controls.

DISCUSSION

More efficient utilization of chlortetracycline (CTC) was obtained when terephthalic acid (TPA) was added to the ration. Terephthalic acid caused no toxic or growth depressing effects in the chicks. Weight gains and feed consumption were normal throughout the 6-week study.

In vivo results with chicks showed that 12.5 grams of CTC per ton of feed plus 0.5 percent TPA was approximately equivalent in activity to 50 grams of CTC without TPA. Furthermore, 25 grams of CTC plus 0.5 percent TPA was as effective as 100 grams of CTC without TPA. If 1.0 percent TPA was mixed into the ration containing 12.5 grams of CTC, infectious synovitis was controlled as effectively as 100 grams of CTC did in the absence of TPA. These results are in close agreement with those reported by Peterson.[3] He reported the blood serum of chicks to have four times more CTC activity when 0.5 percent TPA was present in the ration as when it was not.

There have been two suggestions to explain how TPA enhances the efficacy of CTC. Peterson[3] suggested that a marked improvement in gastrointestinal absorption of CTC occurred. More recently an announcement by Luther[2] suggested that the potentiated antibiotic blood levels could be obtained as a result of reduced renal clearance. Whatever the biochemical action of TPA is, a significant advance toward increasing the efficacy of CTC given in the feed has

resulted. Other tetracycline antibiotics may be potentiated in a like manner.

SUMMARY

Under conditions of this experiment, terephthalic acid greatly enhanced the efficacy of chlortetracycline in controlling infectious synovitis in experimental chicks. As the amount of terephthalic acid in the ration increased from none to 1.0 percent, the minimal amount of chlortetracycline HC1 for control of the disease decreased from 100 to 12.5 grams per ton. This represented a significant advance toward increasing the efficacy of chlortetracycline given in the feed. Although the biochemical action of terephthalic acid remains uncertain, two suggestions have been advanced by others and are included in the discussion.

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THE PROPAGATION AND CYTOPATHOGENIC EFFECT OF AN EGG-ADAPTED STRAIN OF INFECTIOUS BRONCHITIS VIRUS IN TISSUE CULTURE†

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INFECTIOUS bronchitis (IB) is an acute and highly infectious respiratory disease of chickens. Economic losses are due to mortality and retardation of growth in young chicks and loss of production and egg quality in layers. Shalk and Hawn[11] first described the disease and Bushnell and Brandly[2] demonstrated its viral etiology. Beaudette and Hudson[1] propagated the virus in embryonated eggs and Jungherr and Terrell[7] demonstrated parental immunity in chicks. Jungherr *et al.*[8] showed the existence of two antigenically different types of IB virus. Fahey and Crawley[5] propagated IB virus in chicken-embryo tissues and monkey-kidney cells but could not demonstrate a cytopathogenic effect (CPE). Buthala and Mathews[3] could not show any CPE on a single passage of IB virus in chicken-embryo-kidney cells.

In this work the growth of IBV virus in tissue culture was investigated by using serial blind passages in an attempt to produce CPE. This characteristic of the virus would be an added tool in the study of the virus.

Based upon a thesis submitted by the senior author to the faculty of the Graduate School, University of Connecticut, in partial fulfillment of the requirements for the degree of Master of Science, 1958.

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† Presented at the 30th Northeastern Conference on Avian Diseases June 30, July 1 and 2, 1958, Cornell University, Ithaca, New York.

MATERIALS AND METHODS

Virus. Three strains of IB virus were used in this study: the Beaudette egg-adapted strain (No. 66579) now in its 242nd embryo passage, was obtained from Dr. C. H. Cunningham, Michigan State University, East Lansing, Michigan; the Massachusetts strain (No. 82828) obtained from Dr. Henry Van Roekel, University of Massachusetts, Amherst, Mass., has undergone eight bird passages and eight embryo passages; the Connecticut strain (No. A5968) was isolated by one of us (REL) from the trachea of two-week-old birds from the Department of Poultry Science, University of Connecticut, and is now in its 5th embryo passage.

Sera. Normal serum was obtained from known susceptible birds at 6 weeks of age. The birds were inoculated intratracheally with either Connecticut or Massachusetts strain virus and maintained in isolation for 3 weeks when they were bled for antiserum.

Cell Cultures. The method of preparation of cell cultures was a modification of Younger's[12] method for trypsinizing monkey-kidney tissue and growing the cells in a monolayer. The kidneys were removed aseptically from exsanguinated 20-day-old chicken embryos, thoroughly minced with surgical scissors and then washed in Dulbecco's[4] phosphate-buffered saline (PBS) to eliminate excessive blood and debris.

Five ml of 0.25 percent trypsin in PBS, preheated to 40 C, was used for each pair of minced embryo kidneys and stirred in a flask using a Mag-Mix* at a speed to cause good agitation without frothing. Before agitating, the mixture was gently aspirated and released with a warm 10 ml pipette, then agitated for ten minutes, aspirated again, and given a final agitation for 5 minutes. The resulting suspension was filtered through eight layers of 20/12 sterile gauze and centrifuged at 900 rpm for ten minutes, decanted, and the cells resuspended in a medium containing Hanks'[6] basic salt solution (BSS) buffered with 0.5 percent of 7.5 percent sodium bicarbonate and 0.5 percent lactalbumin hydrolysate[9] (LAH) adjusted to pH 7.3 with 4 percent sodium hydroxide. After a second round of centrifuging and decanting in the

* Obtained from Precision Scientific Company, Chicago, Illinois.

above manner, the cells were resuspended in growth medium (Hanks' BBS and LAH + 5 percent bovine serum) at 10 ml per pair of kidneys. A cell count was performed by staining a sample of this suspension with 0.1 percent crystal violet in 0.1 molar citric acid. Growth medium was then added to the suspension to make the final concentration 300,000 cells per ml.

This suspension was distributed in one ml amounts in 16 x 150 mm screw cap Pyrex culture tubes and sealed with white rubber-lined, threaded plastic caps; in 2 ml amounts in Leighton tubes containing 8 x 22 mm glass cover slips; and in ten ml amounts in 3 ounce prescription bottles. The Leighton tubes and prescription bottles were sealed with white rubber stoppers.

Eight to ten-day-old decapitated chicken embryos were used to make cultures of fibroblasts. The tissue was treated as above, using ten ml of trypsin per embryo. A second trypsination was carried out on the residue of the first process, again using ten ml per embryo. Meanwhile the first supernatant was stored temporarily at 4 C. Both supernatants were then mixed, filtered, centrifuged, decanted, etc. as for kidney cells. Hanks' BSS plus LAH and 2 percent bovine serum was used as the growth medium.

The 16 x 150 mm tubes were kept in racks which gave a five degree slant to the tubes; the bottles were layed flat. All cultures were incubated at 37 C. The embryo-kidney cultures required four to five days for a complete sheet to form, at which time the fluid was changed to Hanks' BSS buffered with 1 percent of 7.5 percent sodium bicarbonate plus LAH as a maintenance medium.

Fibroblasts formed a sheet in three days and the fluid was changed to Hanks' BSS buffered with 1 percent of 7.5 percent sodium bicarbonate solution plus 0.1 percent yeast extract as a maintenance medium.

Experimental procedures. In all instances, the amount of inoculum for passage or titration was 0.1 ml per tube and one ml per bottle. For the virus neutralization (VN) test where virus (ten-fold serial dilution) and serum were mixed

in equal parts, the inoculum was 0.2 ml per tube with three tubes per dilution. End points were calculated by the Reed and Muench[10] method. The maximum time the kidney cells were kept after inoculation was four days, the fibroblasts seven to nine days.

After some experience it was observed that severe peeling occurred 2 to 3 days after the sheet of embryo-kidney cells had grown to completion. Therefore the fluid was changed after 3 days growth and the partially complete sheet inoculated. This gave at least some readable part of the sheet for four days.

The inoculum for chicken embryo titration was mixed in Hanks' BSS plus LAH and 0.2 ml was inoculated per embryo via the CAS. For VN tests, a mixture of equal parts of serum and double strength virus dilution (10^{-1}) was inoculated at 0.2 ml per embryo. End points were calculated as above.

Six culture bottles of embryo-kidney cells were inoculated for growth curve determination. At regular intervals, 0.4 ml of infected fluid was removed from each bottle and replaced with 0.4 ml of maintenance fluid.

Infected cultures grown on cover slips were fixed in methyl alcohol, hydrated and stained with hematoxylin and eosin and then mounted permanently on glass slides.

RESULTS

CPE of Beaudette virus on embryo-kidney cells. Undiluted, infected allantoic fluid of both the Connecticut and Massachusetts strains IBV, passed serially five times in embryo-kidney cells, failed to infect chicken embryos.

Allantoic fluid infected with Beaudette IBV produced CPE during the second passage in embryo-kidney cells. The virus caused the cells to undergo even, granular degeneration and their periphery to become extremely distinct at 24 hours. At 48 hours, half the sheet was affected by either granulation or shrinkage; and by 72 hours most of the sheet had either fallen from the glass, or had thinned out into a spider-web-like structure. These progressive effects are shown in figures 1 to 4.

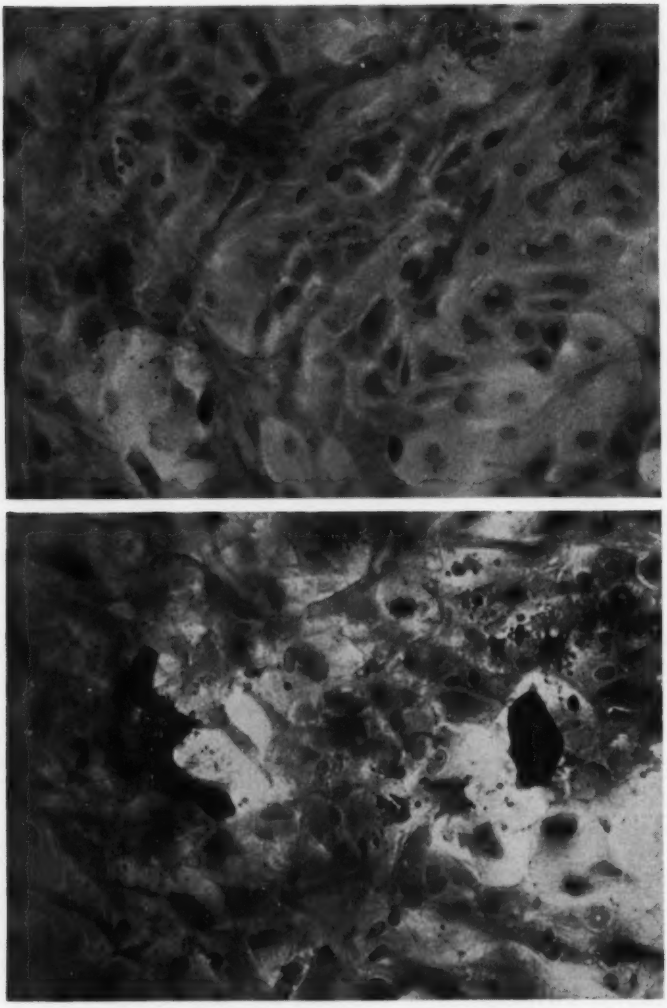

Fig. 1 (top). Normal chicken-embryo-kidney cell culture. Hematoxylin and eosin stain. X200.

Fig. 2 (bottom). Chicken-embryo-kidney cell culture 24 hours after inoculating with IBV. Several cells show granulation. Hematoxylin and eosin stain. X200.

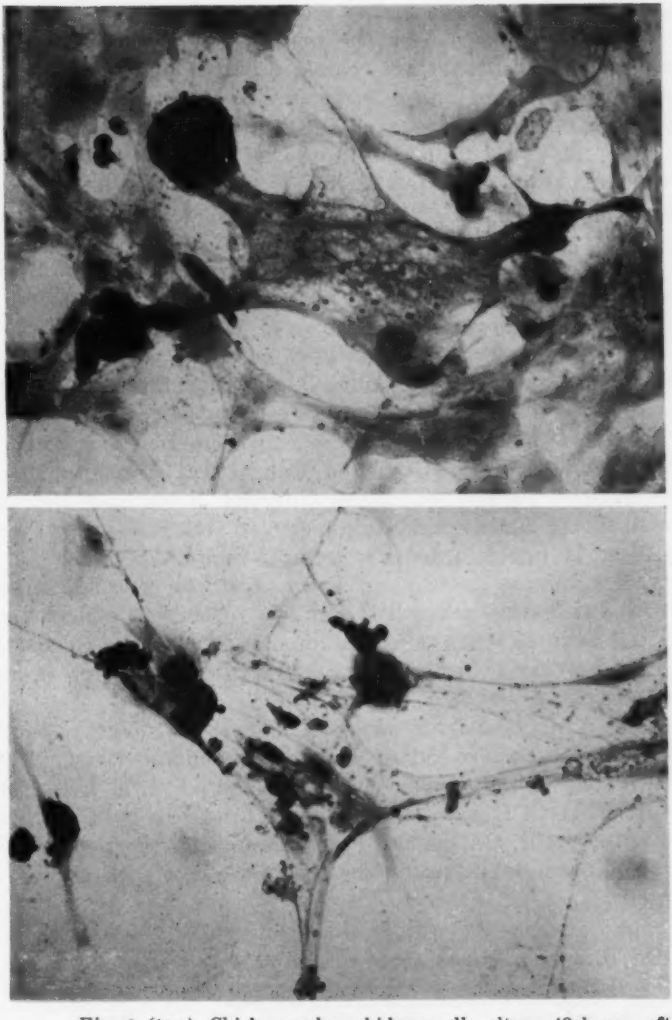

Fig. 3 (top). Chicken-embryo-kidney cell culture 48 hours after inoculating with IBV. Most cells show granulation or shrinkage. Hematoxylin and eosin stain. X200.

Fig. 4 (bottom). Chicken-embryo-kidney cell culture 72 hours after inoculating with IBV. Severe shrinkage of cells has occurred. Hematoxylin and eosin stain. X200.

The fifth passage of the virus titered at $10^{-3.2}$ in tubes, and 10^{-7} in embryos. By the tenth passage the virus titer reached $10^{-4.7}$ in tubes but remained at 10^{-7} in embryos.

Growth curve of the virus in cell cultures. Eighth cell-passage virus, inoculated at 10^{-3}, and titered at various intervals in cell cultures, disappeared from the fluid after 4 hours, reappeared by 16 hours, reached maximum titer at 48 hours where it remained until 72 hours. The titer then declined again. The same samples tested in chicken embryos followed a similar pattern but at approximately 10^{3} times higher infective ability, and the virus remained detectable at all times. The results are shown in figure 5.

Virus neutralization with Connecticut antiserum and Massachusetts antiserum. Undiluted Massachusetts antiserum neutralized 10,000 cell-culture infective doses of tenth passage virus. When the antiserum was diluted 1:10 it lost all neutralizing ability. Undiluted Connecticut antiserum also neutralized 10,000 cell-culture infective doses and lost its neutralizing ability when diluted 1:10. Normal serum neutralized 10 infective doses when used undiluted.

The Massachusetts antiserum and the normal serum showed the same neutralizing ability in chicken embryos as in cell cultures when used undiluted. However, the Connecticut antiserum neutralized only 40 embryo infective doses. The results are shown in table 1.

CPE of Beaudette virus on embryo fibroblasts. Tenth passage on embryo-kidney cells of Beaudette virus, when inoculated undiluted on fibroblasts, caused CPE in a few cells. This CPE was similar to that caused by the virus in kidney cells. On the third passage on fibroblasts the CPE was still not extensive. By the eighth passage CPE appeared extensive.

TABLE I

Virus neutralization doses in chicken embryos and cell cultures

Antiserum	Virus*	
	In cell culture	In embryos
Mass.	10,000	10,000
Conn.	10,000	40
Normal	10	10

* Propagated in chicken-embryo-kidney cell culture.

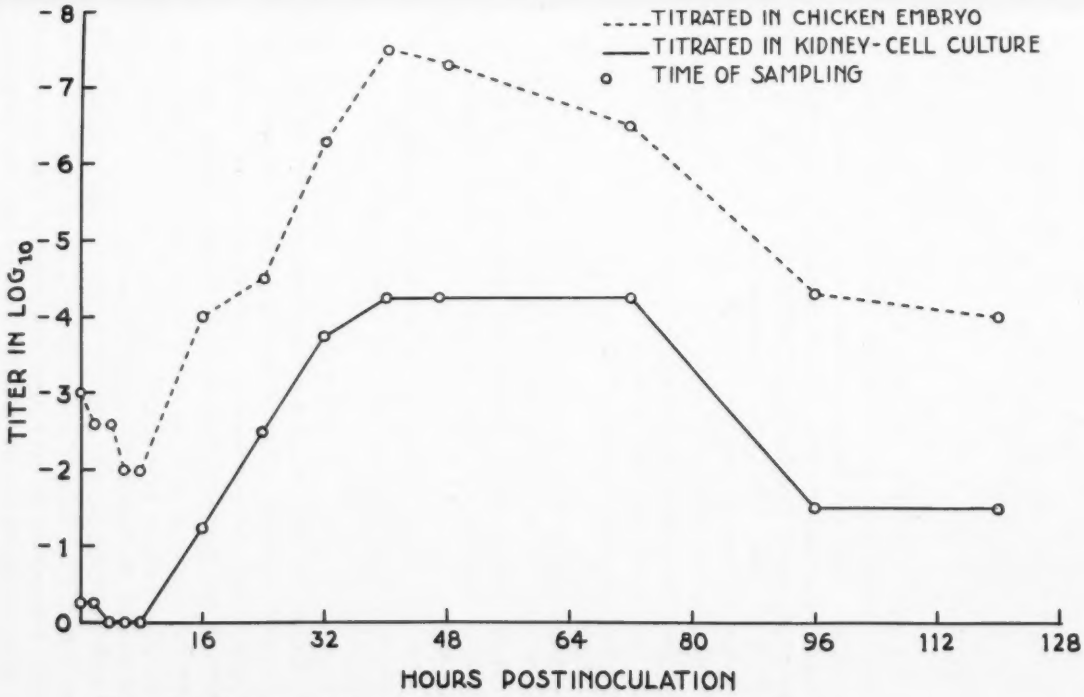

Fig. 5. Growth curves of tissue culture propagated infectious bronchitis virus.

DISCUSSION

The CPE caused by Beaudette IBV on chicken-embryo-kidney cells completely inhibited the metabolism of the tissue. Infected and destroyed cells showed no lowering of pH, while control cells showed this change in two or three days. Since the CPE can be stopped by specific antiserum and not by normal serum, a virus neutralization test is possible. There are two difficulties; the cell sheets peel off at the bottom of the tubes; and undiluted, heat-inactivated chicken serum causes a precipitate to form on the cells, giving them a granular appearance. Despite these difficulties there is a good part of the sheet left in the upper portion of the tube, and CPE can be readily seen.

It was interesting to note that the same cell-passed Beaudette virus was neutralized by both the Massachusetts and the Connecticut type antisera in the cell culture system, but that only the Massachusetts type antiserum neutralized the homologous virus in the chicken-embryo system. The possibility exists that such a relationship plays a role in the antibody mechanism of birds. Antibodies induced by the Connecticut type virus are rarely detected by using the Beaudette egg-adapted virus as antigen in the chicken-embryo VN test.

Further work should be conducted to test the use of chicken-embryo-fibroblasts as the cell culture system for a VN test.

SUMMARY

Beaudette IBV caused a cytopathogenic effect (CPE) in embryo-kidney cells on second passage. The virus caused the cells to shrink or to become evenly granular, and the periphery to become very distinct. By the tenth culture passage the virus titered at $10^{-4.7}$ in cell cultures and 10^{-7} in embryos. The tissue culture propagated virus remained as lethal for embryos as the embryo-propagated virus.

In determining the growth curve in tissue culture it was found that the virus disappeared from the fluid in 4 hours, reappeared in 16 hours, and reached maximum titer at 48 hours, where it remained for 24 hours and then declined.

In cell cultures the tissue culture propagated virus was neutralized by both the Massachusetts and Connecticut type antiserum, but not by normal serum. However the same virus, when tested in embryos, was neutralized only by Massachusetts type antiserum and not by normal serum or Connecticut type antiserum.

Virus passed in embryo-kidney cells caused CPE in chicken-embryo-fibroblast cultures.

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A TISSUE CULTURE-MODIFIED NEWCASTLE DISEASE VIRUS. III. THE IMMUNITY INDUCED BY THE MODIFIED VIRUS AND CRYSTAL VIOLET INACTIVATED VACCINES IN LAYING BIRDS

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THE modification and use of a living tissue culture attenuated Newcastle disease (ND) virus and its immunogenic properties have been previously reported.[1,2] The purpose of the study described herein was to compare this virus with the crystal violet inactivated vaccine produced by the Agricultural Research Service of the USDA. These products were applied to chickens to determine their immunogenicity in a laying flock.

MATERIALS AND METHODS

Chickens. Three hundred one-day-old chicks, obtained from the California Poultry Improvement Commission, were raised in isolation until they reached 11 weeks of age. At this time, the birds were transferred to a previously thoroughly cleaned experimental farm located about three miles from the laboratory. Before vaccination, samples of birds were tested serologically and found to be free of ND antibodies (figure 1), and the chickens were completely susceptible to intramuscular (IM) challenge.

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Vaccines. Tissue culture modified virus vaccine (TC). The vaccine consisted of the supernatant fluid of a 96-hour tissue culture. The titer of the infectious material was determined by inoculating 9-day-old chicken embryonating eggs and was found to be 10^7 per 0.1 ml. The vaccine was prepared by diluting this suspension with Sorenson's phosphate buffered saline (pH 7.2) to contain 12,500 embryo minimum lethal doses (mld) per 0.1 ml. Two passage levels of the virus (77th and 79th) were employed as vaccine.

Crystal Violet Vaccine (CV). The inactivated vaccine was prepared by the Animal Disease and Parasite Research Branch of the Agricultural Research Service of the USDA. One liter of this vaccine, labeled lot No. 3, was furnished for this experiment by Dr. O. L. Osteen. Upon receipt, the vaccine was stored at 4 C for 9 months before use.

Serology. The techniques used for the hemagglutination inhibition (HI) and serum neutralization (SN) tests were described in the previous paper.[1] When sera were pooled for SN determinations, equal volumes of four sera were employed and the sera from the same birds were used for each subsequent pool. In addition, antibody levels at 28, 44, and 64 weeks of age were determined on individual sera of the birds that were challenged. All HI tests were conducted on individual sera. The method of calculating the geometric mean titer was described in a previous paper.[1]

Methods of challenge. Two methods of challenge were employed, the intramuscular (IM) and air borne (AB) routes, using the Texas GB strain of ND virus. This virus was propagated in 9-day-old chicken embryonating eggs, and upon death of the embryos, the allantoic and amniotic fluids were harvested, tested for sterility, and pooled. Enough of the virus was prepared for all challenges, and the infectious material was distributed in 2 ml ampoules and stored at −6 C. Titrations were carried out before each challenge to show that the infectivity titer was stable and to allow a standard dose of virus for each challenge. When given intramuscularly, the virus was diluted in buffered saline to con-

tain 100,000 embryo mld per 0.1 ml.

For the AB route of exposure, the virus suspension was diluted to contain 50,000,000 embryo mld per 0.1 ml and placed in a DeVilbiss No. 40 atomizer. The birds were challenged by placing the beak into the open tube of the atomizer and allowing the bird to inhale the mist for one minute while the bulb of the atomizer was squeezed to its capacity 10 times during the first 45 seconds. For older birds (over 8 weeks of age) an extension of rubber tubing with a 7/8 inch internal diameter was connected to the end of the atomizer to insure an opening large enough to encompass the nares. Past experience had shown that the nares must be well within the tube during inhalation of the mist to obtain sufficient exposure and reproduceable results.

Sample birds were challenged at 15, 20, 29, 47 and 65 weeks of age. In all instances sample birds were removed from the experimental farm and brought to isolation quarters of the laboratory. The isolation rooms were 4 feet by 6 feet and large enough to house five vaccinated chickens and two controls from each experiment. This procedure allowed complete separation of experimental groups from each other and isolation of the IM from the AB challenges. Birds in production were placed in individual cages, bled, and allowed to remain in the environment until egg production was stabilized. It was found that the change from floor to cage confinement was less detrimental to egg production than the bleeding process.

Recovery of virus from tracheas. Ninety-six hours after challenge by either the IM or AB routes, the trachea of each bird was swabbed with sterile cotton attached to a wooden applicator stick, which was immediately placed in 1 ml of veal infusion broth in a test tube. Penicillin and streptomycin at the rate of 5,000 units per ml were added, and the tubes were held at 4 C overnight. This material was inoculated into three 9-day-old chicken embryonating eggs and carried through at least three serial passages in an attempt to isolate the virus.

EXPERIMENTAL DESIGN

When the 300 birds were 11 weeks old, they were divided into groups as shown in table 1, and leg bands to identify each group were affixed. Groups I and III each containing 85 pullets, were vaccinated with the tissue culture attenuated Newcastle disease (TC) and the crystal violet inactivated vaccines (CV) respectively. Twenty-five unvaccinated birds were placed in the units with each of the vaccinated groups as pen contact controls (Ipc and IIIpc) so that the spread of vaccine virus or adventitious infection could be detected. The pens holding the vaccinated birds were separated by a group of 80 susceptible chickens (Group II) which also acted as controls.

When the birds were 20 weeks old they were moved from the brooder house to a floor-operated laying house, which consisted of 5 pens in a row, divided by loose, board partitions. Daily egg production records by pens were maintained throughout the experiment.

Vaccination with TC. Group I was vaccinated intramuscularly in the brooder unit with 0.25 ml of the 77th passage level of TC. The 25 birds held as pen contacts (group Ipc) were in the unit during the procedure but were not handled or vaccinated. Nine weeks later, 60 chickens of group I were moved to a laying house, and in the new quarters the birds were revaccinated intramuscularly with 0.25 ml of the 79th passage level of TC. This group was used to determine the effect of two doses of the vaccine upon the duration and type of immunity produced in laying birds. Thirteen other birds from group I (vaccinated once at 11 weeks of age) were placed in a separate pen of the laying house to determine the duration of immunity to one dose of the virus; this group was identified as group Ia. Nine weeks after the first vaccination, 15 birds of the pen contact control group (Ipc) were challenged to determine whether the vaccine had spread from the vaccinated birds within the same unit, and the remaining 10 chickens were kept in contact with group I in the new quarters.

To further determine the effect of one dose of TC when administered to susceptible 20-week-old birds, 19 house con-

TABLE I

Plan of experiment

Group no.	Number of birds	Treatment	Remarks
In brooder house from 11th to 20th week of age			
I	85 females	Vaccinated TC 11 wks.	6 challenged at 15 and 20 wks of age
Ipc	25 males	Pen contact controls from group I	15 challenged at 20 wks of age
II	25 females 55 males	Nonvaccinated house controls	6 challenged at 15 wks 11 challenged at 20 wks
III	85 females	Vaccinated with CV 1st dose 11 wks of age 2nd dose 15 wks of age	6 challenged at 15 wks and 20 wks
IIIpc	25 males	Pen contact controls for group III	6 challenged at 15 wks 15 challenged at 20 wks
Moved to laying house at 20 weeks of age			
I	60 females	Vaccinated with TC 1st dose 11 wks 2nd dose 20 wks	
Ipc	10 males	Pen contact controls for group I	
Ia	13 females	Vaccinated with TC 1 dose at 11 wks	Portion of group I (Not revaccinated)
IIa	19 females	Vaccinated with TC 1 dose at 20 wks	Portion of group II
IIpc	5 males	Pen contact controls for group IIa	Portion of group II
III	73 females	Vaccinated with CV 1st dose 11 wks 2nd dose 15 wks	
IIIpc	10 males	Pen contact controls for group III	
IV	39 males	Nonvaccinated house controls	Remaining birds of group II

trol birds (group II) were moved into a separate pen of the laying house and vaccinated with 0.25 ml of the 79th passage level of TC and designated as group IIa. Five chickens of group II were left unvaccinated and placed in the same pen to constitute contact controls (group IIpc). At this time 11 birds of group II were challenged to determine whether the virus had spread or if an adventitious infection had occurred during the 9-week period. The remaining 39 birds of group II were maintained as house controls in the new quarters and redesignated as group IV.

Vaccination with crystal violet inactivated vaccine (CV). On the day group I was vaccinated with the TC, each bird of group III was vaccinated intramuscularly with 0.5 ml of the inactivated vaccine. After 4 weeks (when the birds were 15 weeks old) all birds of this group were revaccinated by the same route with 1.0 ml of the vaccine. The 25 pen contact controls (IIIpc) were maintained for detecting an adventitious infection.

Infectious bronchitis immunization. As a precaution against an adventitious IB infection, all birds were vaccinated with a commercial drinking-water IB vaccine at 20 weeks of age.

RESULTS

Reaction of birds to vaccination. Following vaccination with either TC or CV, none of the birds showed clinical reactions. No symptoms involving the respiratory tract or nervous system were observed, and feed consumption was not affected. The second dose of TC, given at 20 weeks of age when egg production was beginning, did not affect or alter the initial egg production and its steady rise. Three days after the second dose of TC, all of the birds were given IB vaccine, which produced the expected respiratory symptoms from the 2nd to the 7th day after vaccination. All of the birds recovered completely from these symptoms. The IB vaccine had no effect on egg production.

Both the house and pen mate controls were present when the TC and CV vaccines were administered. There was no evidence of spread of Newcastle disease from the vaccinated birds to any of the controls during the entire experiment.

Serological response to vaccinations. The serological responses of birds given TC and CV vaccines are shown in figure 1. This figure illustrates the serological responses not only of the vaccinated birds which were picked at random for challenge but also of additional birds from each group which were bled at the intervals shown.

Following the first injection of TC at 11 weeks of age the HI and SN titers rose to a geometric mean (GM) HI titer of 30 or greater and an average log SN titer of 2.4 when tested

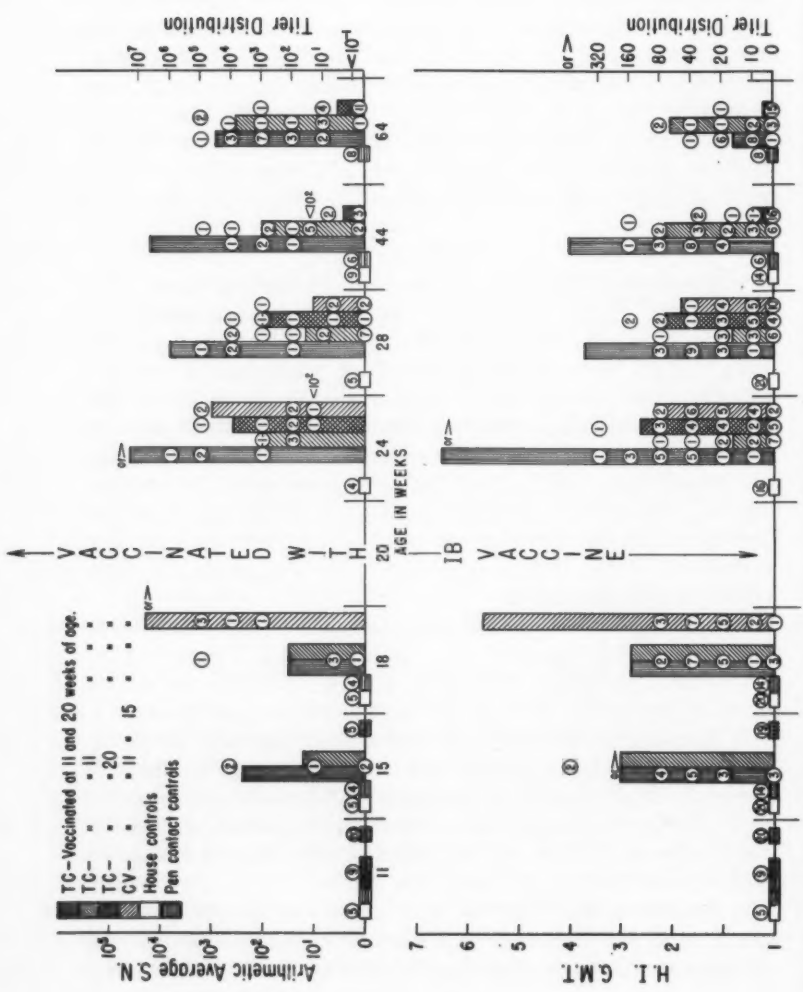

Fig. 1. Serological response to live tissue culture modified (TC) and crystal violet inactivated

at 15 weeks of age (4th week after vaccination). Thereafter the antibodies declined as shown by the results of the test conducted at 18 weeks of age (7th week after vaccination).

Two groups of birds were used to determine the reaction to a single dose of TC. Group Ia, which was part of the original flock vaccinated at 11 weeks of age but not revaccinated, were observed until they reached 28 weeks of age. At this time, (17th week after vaccination) they showed a decrease in HI and SN antibodies, but the birds still presented GM HI titer of 14 and an average log SN titer of 1.2 respectively. Birds of group IIa which were vaccinated for the first time at 20 weeks of age, showed a similar rise in HI and SN antibodies 4 weeks after injection. Significant SN titers were maintained in this group throughout the experiment. The GM HI titer of this group was slightly lower than in group Ia; however, detectable HI antibodies were present for at least 44 weeks after vaccination (table 2).

Revaccination of group I with TC 8 weeks after the first inoculation resulted in an anamnestic response. GM HI and average SN titers increased to 6.5 or greater and 4.75 or greater, respectively 4 weeks later. Although the average SN titer decreased somewhat, significant SN antibodies (10^3 or greater) were maintained for at least 44 weeks following the second dose (table 2). A significantly high GM HI titer was present in the birds at 28 and 44 weeks of age (40 and 57 respectively) that markedly decreased (15) at 64 weeks of age.

Vaccination of chickens with the first dose of CV at 11 weeks of age (group III) did not result in any detectable HI or SN antibodies when the chickens were tested 4 weeks later. Revaccination of the birds at 15 weeks of age resulted in a marked rise in antibody titers. The GM HI and the average SN titers 3 weeks following the second injection (18 weeks of age) were 57 and 4.4 or greater, respectively. These values were greater than those following one injection of TC and approximated the titers attained following revaccination with the live virus (figure 1). Continued testing of the sera of this group when the birds reached 24, 28, 44, and

Fig. 1. Serological response to live tissue culture modified (TC) and crystal violet inactivated (CV) Newcastle vaccines

TABLE II

Distribution of HI and SN titers of TC and CV vaccinated birds at 28, 44 and 64 weeks of age

		Age Tested									
		28 Weeks				44 Weeks			64 weeks		
	Treatment Group No.	TC2[1] I	TCIE[2] Ia	TCIL[3] IIa	CV[4] III	TC2 I	TCIL IIa	CV III	TC2 I	TCIL IIa	CV III
SN Titers[5]	<1 or <2	1	7	1	7	0	1	3	0	1	13
	1	0	2	1	4	0	5	10	2	3	6
	2	2	1	1	1	2	1	0	4	1	1
	3	5	1	1	1	3	2	2	8	1	1
	4	3	2	1	1	6	1	0	5	1	0
	5	2	0	0	1	1	1	0	1	2	0
	6	0	0	0	0	1	0	0	0	0	0
	7	0	0	0	0	1	0	0	0	0	0
		3.5	1.2	2.0	1.0	3.0	2.4	0.6	3.1	2.4	0.6
HI Titers[7]	Average SN Titer[6] 0	1	6	4	14	0	5	25	7	3	57
	10	1	3	5	9	1	2	1	15	2	0
	20	4	3	3	4	5	2	1	13	1	2
	40	14	0	1	1	8	3	2	5	1	0
	80	4	1	2	2	6	2	0	1	2	0
	160	2	0	1	0	4	1	0	0	0	0
	320 or >	0	0	0	0	2	0	0	0	0	0
	HI GMT	40.	14.	19.	13.	57. or>	17.	11.	15.	20.	10.

[1]TC2—Tissue culture vaccine at 11 and 20 weeks of age.
[2]TCIE—Tissue culture vaccine at 11 weeks only.
[3]TCIL—Tissue culture vaccine at 20 weeks only.
[4]CV—Crystal violet vaccine at 11 and 15 weeks of age.
[5]Expressed as log exponent.
[6]In calculating the arithmetic average, the pooled samples were weighed in relation to number of individual samples in pool. See Fig. 1 for number pools and individual samples.
[7]Expressed as reciprocal of titer dilution.

64 weeks of age showed a progressive decrease in both HI and SN antibody titers. This decrease was considerably more rapid in birds vaccinated with TC. It was noted that HI and SN titers at 28, 44, and 64 weeks of age were below values considered significant.

None of the control groups (Ipc, IIpc, and IIIpc) that were in direct contact at all times with the birds vaccinated with the TC and CV showed evidence of infection during ex-

posure to the vaccinated chickens. HI and SN antibodies were not found in their sera at any time, and birds of each group were readily susceptible to a challenge dose of ND virus throughout the experiment. In addition, the house controls (group II), which were held in a pen situated between the vaccinated groups, showed no serological evidence of infection with NDV. A portion of this group when vaccinated at 20 weeks of age (group IIa) responded serologically similarly to other groups of birds (group I and group Ia) given the vaccine for the first time. The absence of an anamnestic response in group IIa was further evidence that a previous exposure to the NDV did not occur during this observation period.

The data of the serological responses are presented in figure 1 on a flock basis. Table 2 compares the distribution and persistence of the titers incited by the TC and CV vaccines in individual and pooled sera during the latter stages of the experiment. It can be seen from these data that the TC-vaccinated birds not only maintained a greater range and higher titers of HI and SN antibodies at 28, 44 and 64 weeks of age but that detectable titers persisted in a higher percentage of the birds tested at these times. This persistence of detectable HI and SN antibodies were found not only in birds given two doses of TC but also in the group which received one dose of TC. In comparison, relatively few individuals vaccinated with CV maintained detectable HI and SN antibodies in the sera at 44 and 64 weeks of age.

Results of challenge with virulent NDV. Birds picked at random from each of the groups were removed to separate quarters at a distance from the experimental flock for challenge by the IM and AB routes to determine the degree of immunity induced by the vaccines. Such challenges were conducted at 15, 20, 29, 47 and 65 weeks of age. The discrepancy between the time of the serological and challenge results was due to the necessity to acclimatize the birds to the new environment, particularly the laying birds. In each case the interval between dates of bleeding and challenge is noted in the appropriate table. The results of challenge are shown in tables 3, 4, 5, 6, and 7.

TABLE III

Results of challenge by intramuscular (IM) and air borne (AB) routes at 15 weeks of age

Vaccination history	Route of challenge	No. of birds	HI GMT†	SN avg.‡	Recovery of virus*	Clinical symptoms§
TC 1st dose 11 wk (group I)	IM	3	30 or>	2.4	3 birds on 2nd	3 R
	AB	3			2 birds on 2nd	3 R
CV 1st dose 11 wk (group III)	IM	3	All Neg	<10	3 birds on 2nd	3 R
	AB	3			3 birds on 2nd	3 R
Nonvaccinated house controls (group II)	IM	3	All Neg	<10	3 birds on 1st	3 S
	AB	3			1 bird on 1st	3 R

† Value of twenty birds bled at random from group.
‡ Results of 5 pools of sera from 20 birds bled at random from group.
* Number of passages required to isolate virus. None = no virus isolated in 3 serial passages.
§ R = refractory; Rr = mild rales only; S = paralysis and death.

Symptoms and mortality after challenge. All of the control birds were susceptible to IM challenge. Except for challenges at 15 and 20 weeks of age, all control birds challenged by the AB route were susceptible. The failure of these two AB challenges was due to faulty apparatus. All susceptible birds developed typical respiratory and nervous symptoms which were followed by death in a few days.

Only three vaccinated birds were susceptible to challenge. Following a single dose of TC one bird at 20 weeks of age (9th week after vaccination) succumbed to IM challenge (tables 4 and 5). One other bird given two doses of CV died at 65 weeks of age, 50 weeks after vaccination (table 7). No other vaccinated birds succumbed to challenge throughout the experiment.

None of the birds challenged intramuscularly with virulent NDV developed respiratory symptoms; however, some of the birds developed rales after air-borne exposure to the virus. There was no consistent correlation between the vaccination history of the birds and the development of audible rales following the air-borne challenge. Generally, more birds

TABLE IV

Results of challenge by intramuscular (IM) and air borne (AB) routes at 20 weeks of age

Vaccination history	Route of challenge	No. birds	HI GMT†	SN avg.‡	Recovery of virus*	Clinical symptoms§
TC 1st dose 11 wks (group I)	IM	3	28	1.6	1 bird on 1st	2 R; 1 S.**
	AB	3			None	3 R
CV 1st dose 11 wks 2nd dose 15 wks (group III)	IM	3	57.	4.5 or>	None	3 R
	AB	3			1 bird on 1st	3 R
Nonvaccinated house controls (group II)	IM	3	All Neg	<10	3 birds on 1st	3 S
	AB	3			None	3 R
Nonvacc. penmates (group Ipc)	IM	15	All Neg	<10	Not Done	15 S
Nonvacc. penmates (group IIIpc)	IM	15	All Neg	<10	Not Done	15 S
Nonvaccinated house controls (group II)	IM	5	All Neg	<10	Not Done	5 S

† Sera for HI and SN taken 12 days prior to challenge of group. HI results of 20 birds bled at random from group.

‡ Results of 5 pools of sera of 20 birds bled at random from group.

* Number of passages required to isolate virus. None = no virus isolated in 3 serial passages.

§ R = refractory; Rr = mild rales only; S = paralysis and death.

** Same bird.

given two doses of CV or only one of the TC developed symptoms than those given two doses of TC. Furthermore, more birds developed rales in all three groups as the experiment progressed (tables 5, 6 and 7).

Reisolation of virus from tracheas. As another measure of the immunity induced by vaccination, the susceptibility of the respiratory tract to IM and AB challenge was tested by determining whether the virus could be reisolated from the trachea 96 hours after exposure. An additional criterion con-

TABLE V

Results of challenge by intramuscular (IM) and air borne (AB) routes at 29 weeks of age

Vaccination history	Route of chal.	No. of birds	HI†	SN†	Recovery of virus‡	Clinical symptoms*	Effect on egg production		
							Pause	Duration of pause days	External abnorm.
TC 1st dose 11 wks 2nd dose 20 wks (group I)	IM	5	1 – 0 1 .. 20 3 – 40	1 – <1 3 .. 3 1 – 4	None	5 – R	0/5	—	1/5
	AB	5	2 – 40 1 – 80 2 – 160	1 – 2 2 – 3 1 – 4 1 – 5	5 birds on 1st	3 – R 2 – Rr	2/5	1) 4-28 2) 4-11	0/5
TC 1 dose 11 wks (group Ia)	IM	5	2 – 0 2 – 10 1 – 20	3 – <1 1 – 1 1 – 2	2 birds on 1st 1 bird on 3rd	4 – R 1 – S	1/4	1) 6-16	0/4
	AB	8	4 – 0 1 – 10 2 – 20 1 – 80	4 – <1 1 – 1 1 – 3 2 – 4	8 birds on 1st	8 – Rr	8/8	1) 6-15 2) 9-15 3) 18-23 4) 6-17 5..7-18 6) 5-12 7) 6-4	0/8
CV 1st dose 11 wks 2nd dose 15 wks (group III)	IM	5	1 – 0 3 – 10 1 – 80	1 – <1 2 – 1 1 – 2 1 – 4	None	5 – R	1/5	1) 22-39	1/5
	AB	5	3 – 0 1 – 10 1 – 80	4 – <1 1 – 5	5 birds on 1st	3 – R 2 – Rr	4/5	1) 5-13 2) 4-16 3) 7-15 4) 6-22	3/5
None house controls (group IV)	IM	3	3 – 0	3 – <1	3 birds on 1st	3 – S			
	AB	5	5 – 0	5 – <1	5 birds on 1st	5 – S			

(group IV)	AB	5	5 – 0	5 – <1	5 birds on 1st	5 – S
None	IM	6	6 – 0	6 – <1	6 birds on 1st	6 – S
virus challenge controls	AB	6	6 – 0	6 – <1	6 birds on 1st	6 – S

† Sera collected 6 days prior to challenge. Number of birds having titer expressed as reciprocal of log (SN) or doubling dilution (HI) of serum titer.

‡ Number of passages required to isolate virus. None = no virus isolated in 3 serial passages.

* R = refractory; Rr = mild rales only; S = paralysis and death.

sidered was the number of passages through chicken embryonating eggs required to isolate the virus.

The degree of resistance of the respiratory tract to AB challenge when the birds were 15 and 20 weeks old could not be evaluated because the controls did not respond to challenge at these times (tables 3, 4). The degree of resistance of the respiratory tract of vaccinated birds to AB challenge at 29, 47, and 65 weeks of age could not be measured either because all of the vaccinated and control chickens yielded the virus from the trachea on the first passage.

Differences in the readiness with which virus could be isolated from the tracheas of the various groups of birds were observed following IM challenge. The virus was isolated on first passage from all the nonvaccinated control chickens. It was less readily reisolated from the vaccinated birds which had received one dose of TC and CV vaccines than from the controls when these birds were challenged at 15 weeks of age (table 3).

In birds challenged at 20 weeks of age, an even greater difference was noted since the virus was isolated from only one bird which received one dose of TC 9 weeks previously and from none of the birds which received two doses of CV (table 4).

After the birds were in production and challenged intramuscularly at 29 weeks of age, the virus could not be isolated from the tracheas of the birds given two doses of either TC or CV. In the group given one dose of TC 18 weeks previously, three of the five birds yielded the virus by the second passage.

Following IM challenge at 47 weeks of age, NDV was isolated from the tracheas of all nonvaccinated controls on the first passage but was not readily recovered from the vaccinated birds. With chickens given one dose of TC (group IIa) 27 weeks previously, the virus was recovered on first passage from the tracheas of three of the four birds tested. The remaining birds yielded the virus on second passage. The virus was readily isolated from four of the five birds given two doses of the crystal violet vaccine (group III), but no

virus was isolated from the fifth bird. The greatest degree of resistance of the trachea to the virus was found in group I which received two doses of TC. No virus was isolated from three of the five birds in three serial passages through embryonating eggs, but it was recovered from the fourth bird on the initial passage and from the fifth on the second passage.

When the birds reached 65 weeks of age and were challenged by the IM route, NDV was readily isolated from the tracheas of all of the nonvaccinated controls (Ipc, IIpc, IIIpc and IV). Of four chickens given one dose of TC 45 weeks previously (group IIa), two yielded the virus. The NDV was isolated from the tracheas of six of the eight birds given two doses of CV (group III) but from only one of the eight birds given two doses of TC (group I).

Effect of challenge on egg production. To determine the effect of challenge on egg production, birds were kept in individual laying cages. Careful daily observations and records of egg production were made during the acclimatization period and for at least 35 days after challenge.

The effect of challenge on egg production at 29 weeks of age is shown in table 5. IM challenge caused no significant difference in egg production of the three groups of vaccinated birds (I, Ia and III); only one bird in each of the two groups (I and Ia) given TC showed either a pause in production or an externally abnormal egg. Group III which received two doses of CV presented one bird which ceased egg production and one chicken which laid eggs of abnormal appearance.

Marked differences between groups in egg production and quality were noted following AB challenge. Group I, which received two doses of the TC, showed a better protection against a drop in egg production, as only two of the five birds tested paused. All eight birds in group Ia, which received one dose of the vaccine 17 weeks previously, showed a drop in egg production which ranged from 5 to 11 days. In comparison, four of the five birds vaccinated with two doses of CV were out of production for periods of 8 to 16 days. It is to be noted that while none of the birds given one or two doses of TC produced any externally abnormal eggs after AB challenge, three

TABLE VI

Results of challenge by intramuscular (IM) and air borne (AB) routes at 47 weeks of age.

Vaccination history	Route of chal.	No. of birds	HI†	SN†	Recovery of virus‡	Clinical symptoms*	Effect on egg production		
							Pause	Duration of pause days	External abnorm.
TC 1st dose 11 wks 2nd dose 20 wks (Group I)	IM	5	2 – 40 1 – 80 1 – 160 1 – >320	3 – 4 1 – 6 1 – 7 or>	1 bird on 1st 1 bird on 2nd	5 – R	1 Molt 0/4	1) 7→	0/4
	AB	5	1 – 10 1 – 20 2 – 80 1 – 160	1 – 2 1 – 3 2 – 4 1 – 5	5 birds on 1st	5 – R	5 Molt	1) 1→ 2) 2→ 3) 4→ 4) 4→ 5) 4→	
TC 1st dose 20 wks (group IIa)	IM	4	2 – 0 1 – 20 1 – 40	3 – <2 1 – 5	3 birds on 1st 1 bird on 2nd	4 – R	1 Molt 0/3	1) 4→	0/3
	AB	4	2 – 0 1 – 10 1 – 20	3 – <2 1 – 3	4 birds on 1st	4 – Rr	4 Molt	1) 4-17 2) 3-19 3) 4→ 4) 3-23	
CV 1st dose 11 wks 2nd dose 15 wks (group III)	IM	5	5 – 0	4 – <2 1 – 3	4 birds on 1st 1 bird on 2nd	5 – R	2 Molt 0/3	1) 8→ 2) 5→	0/3
	AB	5	4 – 0	4 – 2 or< 1 – 3	5 birds on 1st	5 – Rr	4/5	1) 4-17 2) 7-20 3) 4-13 4) 4-17	0/5

Nonvaccinated penmates (one each of Groups Ipc, IIpc and IIIpc)	IM	3	3 – 0	3 – <2	3 birds on 1st	3 – S
	AB	3	3 – 0	3 – <2	3 birds on 1st	3 – S
Nonvaccinated house controls (group IV)	IM	2	2 – 0	2 – <2	2 birds on 1st	2– S
	AB	2	2 – 0	2 – <2	2 birds on 1st	2– S

† Sera collected 23 days prior to challenge. Number of birds having titer expressed as reciprocal of log (SN) or doubling dilution (HI) of serum titer.

‡ Number of passages required to isolate virus. None = no virus isolated in 3 serial passages.

* R = refractory; Rr = mild rales only; S = paralysis and death.

TABLE VII

Results of challenge by intramuscular (IM) and air borne (AB) routes at 65 weeks of age

Vaccination history	Route of chal.	No. of birds	HI†	SN†	Recovery of virus‡	Clinical symptoms*	Effect on egg production		
							Pause	Duration of pause days	External abnorm.
TC 1st dose 11 wks 2nd dose 20 wks (group I)	IM	8	1 – 0 4 – 10 2 – 20 1 – 40	1 – 1 2 – 2 2 – 3 2 – 4 1 – 5	1 bird on 2nd	8 – R	2/8	1) 10→ 2) 9→	0/8
	AB	8	4 – 10 4 – 20	1 – 1 1 – 2 5 – 3 1 – 4	8 birds on 1st	8 – Rr	5/8	1) 4→ 2) 4→ 3) 4→ 4) 5-22 5) 8-17	0/8
TC 1st dose 20 wks (group II)	IM	4	2 – 0 1 – 20 1 – 80	1 – <1 1 – 1 1 – 3 1 – 5	2 birds on 1st	4 – R	2/4	1) 7-18 2) 5-22	1/4
	AB	5	1 – 0 2 – 10 1 – 40 1 – 80	2 – 1 1 – 2 1 – 4 1 – 5	5 birds on 1st	3 – R 2 – Rr	3/5	1) 5→ 2) 6→ 3) 18→	0/8
CV 1st dose 11 wks 2nd dose 15 wks (group III)	IM	8	8 – 0	5 – <1 3 – 1	5 birds on 1st 1 bird on 2nd	1 – S 7 – R	2/7	1) 5-21 2) 8-22	2/8
	AB	8	7 – 0 1 – 20	6 – <1 1 – 1 1 – 3	8 birds on 1st	8 – Rr	8/8	1) 6-28 2) 5-20 3) 8-18 4) 6-16 5) 7-17 6) 5-17 7) 5-24 8) 6-17	0/8

Nonvaccinated pen contacts (1 each of groups Ipc, IIpc & IIIpc	IM	3	3 – 0	3 – <1	3 birds on 1st	3 – S
	AB	3	3 – 0	3 – <1	3 birds on 1st	3 – S
Nonvaccinated house controls (group IV)	IM	4	4 – 0	4 – <1	4 birds on 1st	4 – S
	AB	4	4 – 0	4 – <1	4 birds on 1st	4 – S

† Sera collected 12 days prior to challenge. Number of birds having titer expressed as reciprocal of log (SN) or doubling dilution (HI) of serum titer.

‡ * See footnotes Table V.

of the five birds given two doses of CV did produce externally abnormal eggs. In all cases these eggs were obtained immediately after the pause.

The quantitative differences of the effect of AB challenge on these vaccinated groups are presented in figure 2 which presents the data on marketable eggs only. This figure demonstrates the less severe effect and more rapid return to normal production in chickens given two doses of TC than in the groups given the CV. Furthermore, the group vaccinated once with TC showed as great a drop in production as did the group given two doses of CV, but the TC-vaccinated group returned to production much more rapidly.

Effect of challenge on egg production at 47 weeks of age is shown in table 6. Thirteen of the 28 birds went into a molt which prevented an interpretation of the effect of challenge on egg production. It can be seen that the molting process and cessation of egg production appeared sooner than the drop in production that might be expected with ND infection as observed in the previous challenge.

The effect of challenge on egg production at 65 weeks of age is shown in table 7. These data confirm the observations made at the 28th-week challenge that the difference in egg production was less significant with IM than with AB challenge. Only two birds from each of groups I, IIa and III paused or ceased egg production following IM challenge. On the other hand, all eight of the CV-vaccinated birds (group III), 5 of 8 birds vaccinated twice with the TC vaccine (group I), and 3 of 5 birds vaccinated once with the TC vaccine (group IIa) paused in egg production following AB challenge. Generally, the pause started 4 to 8 days after challenge. The pause in birds given two doses of TC varied from 9 to 31 days, the latter being the longest period observed. The pause in the group receiving the crystal violet vaccine ranged from 10 to 22 days.

No externally abnormal eggs were laid by the birds given two doses of TC (group I). One bird from the group given one dose of the TC vaccine 45 weeks previously and 2 of the birds given two doses of CV (group III) laid abnormal eggs following IM challenge.

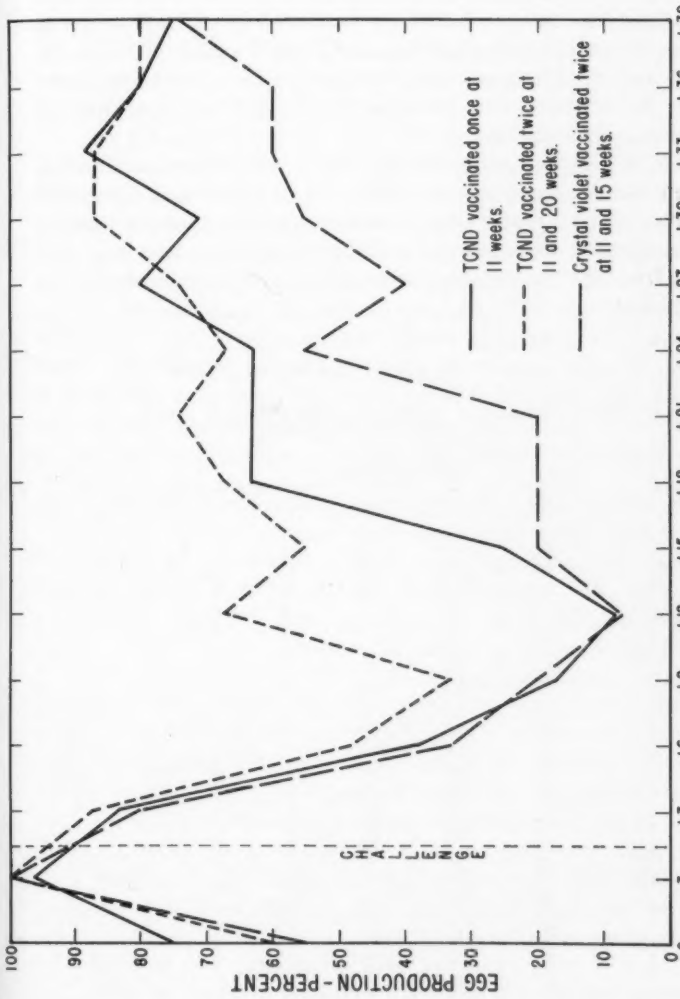

Fig. 2. A comparison of the effect of airborne challenge at 29 weeks of age on egg production of chickens vaccinated with TC and CV.

Relationship of serology and challenge results. An analysis of the data obtained from individual birds revealed a lack of correlation between serological and clinical manifestations of the disease following challenge by the IM and AB routes. Since the data in tables 5, 6, and 7 could not be tabulated on an individual bird basis, the data were extracted from these tables and compiled in table 8 to illustrate the following observations.

A. Following AB challenge there was no correlation between the presence of significant HI and SN titers and the development of respiratory symptoms in the form of audible rales (birds 1 through 8). In this group the virus was isolated from the tracheas of birds with and without respiratory symptoms.

TABLE VIII

The correlation of serological and challenge results in selected individual birds

Group No.		Age at challenge in weeks	Route of challenge	HI titer	SN† titer	Respiratory symptoms	Virus isolation	Pause in egg productic
1	IIa	65	AB	80	5	+	+	0
2	I	29	AB	40	3	+	+	+
3	I	29	AB	80	5	0	+	0
4	IIa	65	AB	40	4	0	+	+
5	III	29	AB	0	<1	+	+	+
6	III	29	AB	10	<1	+	+	+
7	III	29	AB	0	<1	0	+	+
8	IIa	65	AB	0	1	0	+	+
9	1	29	AB	160	3	+	+	+
10	Ia	29	AB	80	4	+	+	+
11	III	29	AB	80	5	0	+	0
12	I	29	AB	160	4	0	+	0
13	I	47	IM	>320	<7	0	+	0
14	I	47	IM	80	4	0	+	0
15	I	47	IM	40	4	0	0	0
16	I	47	IM	40	4	0	0	0
17	III	29	IM	10	1	0	0	+
18	III	65	IM	0	1	0	0	0
19	III	47	IM	0	<2	0	+	0
20	III	47	IM	0	<2	0	+	0
21	I	29	IM	20	4	0	0	0
22	I	29	IM	40	3	0	0	0
23	I	29	IM	0	<1	0	0	0
24	I	65	IM	0	1	0	0	0

† SN titer expressed as – log of serum dilution.

B. There did not appear to be a correlation between the presence of significant HI and SN titers and isolation of ND virus from the tracheas of birds challenged by the air-borne route.

C. The relationship between HI and SN titers and the effect of challenge on egg production appeared to differ with the route of challenge.

All birds with SN titers of 10^3 or greater resisted a drop in egg production when challenged intramuscularly. However, many birds with SN titers of 10^3 or less also resisted a drop in egg production when this route of challenge was used. All birds that ceased egg production following IM challenge had SN titers of 10^2 or less. Birds challenged by the air-borne route did not show the same correlation. In this case birds with SN titers as high as 10^4 ceased egg production, and all birds that did not pause had titers of 10^3 or greater. In addition to the examples shown in table 7 birds showing these correlations can be found in tables 5, 6, and 7.

DISCUSSION

Inoculation of birds with the living tissue culture modified virus (TC) did not induce any respiratory or nervous symptoms of Newcastle disease or other untoward effects. In addition to this advantage, the virus did not spread to susceptible chickens kept in direct contact with vaccinated birds for 54 weeks. These observations of the behavior of TC confirm the results of previous experiments conducted in younger birds.[2] Since TC virus does not spread from bird to bird, it can be used safely in the presence of susceptible birds. Because it does not cause any detectable reaction, it is not likely to influence the course of an infection present in the flock or to aggravate symptoms due to other live-virus vaccines. Furthermore, these characteristics suggest that this virus can be more safely used in conjunction wth other live-virus vaccines than the current live-virus Newcastle disease vaccines. The TC can be used, therefore, with the same degree of safety as the inactivated CV.

The serological response produced by vaccination with

TC was superior to that induced by CV. TC inoculations produced higher and more persistent HI and SN titers than CV (figure 1, table 2). Birds vaccinated with CV showed high HI and SN titers approximately 4 weeks after the second dose. Thereafter the antibody titers rapidly and progressively decreased, dropping below significant values when the birds were tested at 28, 44, and 64 weeks of age. Birds vaccinated with TC developed high antibody titers which persisted for a considerable period. Significant average SN titers were still maintained when the birds were 64 weeks of age. This was observed in the groups receiving one dose of TC at 20 weeks of age and in the group receiving two doses one at 11 and one at 20 weeks of age. High GM HI titers were found in birds given two doses of TC. These titers persisted until the birds were 44 weeks old and decreased when tested at 64 weeks of age. Following a single dose of TC at 20 weeks of age the initial HI response was considerably lower and gradually decreased. However, HI antibodies were detectable in most of the birds given one or two doses of TC at the end of the experiment (table 2). In no instance were HI or SN antibodies detected in any of the controls, and all controls were susceptible to challenge. This indicated that there was no adventitious exposure or spread of virus from one bird to another. The immune response of the vaccinated groups is, therefore, attributable only to the vaccines.

Only three birds, one vaccinated with two doses of CV (50 weeks after first vaccination) and two vaccinated with one dose of TC (one at 9 and one at 18 weeks after first vaccination) succumbed to IM challenge. None of the chickens given two doses of TC succumbed. Both vaccines, therefore, were considered very effective in protecting chickens against death when exposed to either IM or AB challenge.

The results of this experiment indicate that the respiratory symptoms following challenge were not a useful measure of immunity. None of the birds challenged by the IM route developed audible respiratory symptoms, while a considerable number of those challenged by the AB route did develop rales. There was no correlation between the development of

rales and any other criterion employed (reisolation of the virus from trachea, serological response, mortality, and egg production).

The virus was isolated from the tracheas of all vaccinated and control birds following AB challenge at 29, 47, and 65 weeks of age. The isolations were accomplished regardless of the HI or SN antibody levels. Differences in the degree of protection of the respiratory tract were noted in birds challenged by the IM route. These differences could not be measured at 29 weeks of age, but two doses of TC gave the respiratory tract significantly better protection when the birds reached 47 and 65 weeks of age than two doses of CV.

The immunity induced by these vaccines was evaluated according to serological response, susceptibility of respiratory epithelium, mortality, and egg production following IM and AB challenge. It was immediately noted that not only did the results vary with the route of challenge but also the effects of challenge revealed no correlation between these different criteria. In individual birds challenged by the AB route, high HI and SN titers were not correlated with protection of the respiratory and reproductive systems.

Following IM challenge, birds with both high and low antibody titers showed no drop in egg production and considerable protection of the respiratory epithelium; these results were more evident in birds receiving two doses of TC than in birds receiving two doses of CV.

The effect of challenge on egg production was observed when the birds were 29, 47 and 65 weeks of age. Following challenges at 29 weeks of age, significant differences in egg production between groups were noted. While IM challenge had relatively little effect on egg production, differences in reaction to AB challenge were greater and more readily discernible. The capacity to lay eggs was best protected with two doses of TC. Fewer birds paused in egg production and no abnormal eggs were produced. Furthermore, this group (I) showed a less severe slump and a more rapid return to normal production than the group receiving two doses of CV. Unfortunately it was not possible to use egg-production data

of the challenge conducted at 47 weeks of age. Most of these birds molted and ceased egg production. This effect on egg production was apparently due to other causes than challenge, since the birds went out of production sooner than would be expected as a result of virus challenge. A similar molt and drop in egg production occurred in birds that were not challenged. Challenge at 65 weeks of age showed some protection induced by the two doses of TC, while all of the birds vaccinated with CV paused following challenge. In view of the significant differences between the two vaccines as judged by other criteria, this difference in egg production was less than expected. This leads us to believe that factors other than the challenge virus were responsible for these effects on egg production. Egg production under laboratory conditions should perhaps be studied on a flock or pen basis with larger groups of birds and without introducing factors of movement and changes of environment. It is also realized that the results might have been more satisfactory if completely susceptible laying controls had been available for comparison. It is possible that a more adequate test of the TC-induced immunity to protect against drop in egg production can be made with another group of birds presently being studied on a commercial egg ranch under field conditions.

As was pointed out in the results, there was little correlation between the serological titers (HI and SN) and the degree of protection of the respiratory tract, reproductive system and protection against the systemic reaction to infection. This was further complicated by the variation in the response of the vaccinated birds to the two routes of challenge. Although the influence of strain of virus, dosage, exposure and route of challenge are known to be important factors in measuring an immunity, it must be conceded that with neither route did the reaction to challenge simulate a natural infection.[3,4,5] Thus neither method was satisfactory in measuring all of the factors of immunity. IM inoculation must be regarded as an unnatural route of exposure, and the dosage and length of exposure used in the AB method may have been overwhelming.

Perhaps there is no correlation between detectable serum antibodies and protection of the respiratory epithelium and reproductive system. Some vaccinated birds with no detectable antibodies were resistant to IM challenge. Further, some birds with high antibody levels (considered significant) were susceptible to a drop in egg production and also showed a susceptibility of the respiratory epithelium (table 8). On the other hand, some birds with high antibody titers did not show this susceptibility of respiratory and reproductive system. This clearly indicates that there are factors concerned with the immunity of the respiratory and reproductive systems which were demonstrated by the other methods used to test immunity but which are not reflected in the detectable serum antibodies (HI and SN).

Varying degrees of susceptibility of the respiratory and reproductive systems, observed in vaccinated birds that were not killed by challenge doses of virulent NDV that killed 100 percent of the controls, suggest that the respiratory and reproductive tracts may be independent of systemic immunity, and possibly of each other, in their susceptibility to infection. The independence of behavior of the various immunity factors discussed in this report shows the complexity of the immune response to ND virus.

SUMMARY AND CONCLUSIONS

A tissue culture modified Newcastle disease virus (TC) administered intramuscularly into 11-week-old chickens gave a significant immunity without producing symptoms or any untoward reactions. Two doses of TC given 9 weeks apart produced significantly higher and more persistent serum antibodies than did two doses of crystal violet inactivated ND vaccine (CV). Both vaccines gave good protection against nervous symptoms and death following intramuscular (IM) and air-borne (AB) challenge with the GB strain of NDV for 45 and 50 weeks after the second dose of TC and CV vaccines respectively. Neither house controls nor pen contact controls developed antibodies or a resistance to challenge throughout a 54-week period. Thus there was no evidence of

spread of the living-vaccine virus from the vaccinated to susceptible contact controls during this period.

The immunity of the respiratory and reproductive tracts following vaccination with TC and CV was tested by several methods, using both IM and AB routes of challenge. The lack of correlation of these results with the HI and SN antibody titers in sera of individual birds is discussed in detail.

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A FURTHER INDICATION OF EGG TRANSMISSION OF INFECTIOUS SYNOVITIS*

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A DISEASE entity since termed infectious synovitis was independently reported by Olson[1] and Wills[5] in 1954. The following year it was reported as a naturally occurring disease of turkeys.[3] In 1955 Wills[5] reported the development

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of the disease at 21 days of age in 1 of 1470 progeny of breeding chickens exposed to infectious synovitis. He interpreted these results as highly suggestive but not conclusive evidence of egg transmission. The same report summarized a field observation which suggested egg transmission of the agent. The disease has developed in chicks hatched from eggs inoculated with the agent.[2,5] Thayer *et al.*[4] reported finding lesions of the hocks in unhatched 21-day-old chicken embryos and in 1 and 6-day-old chicks from commercial hatcheries. The disease was reproduced from the 1 and 6-day-old chicks. Lesions were produced following inoculation on the chorioallantoic membrane of material from the unhatched embryos; infectious synovitis was not reproduced in chicks injected with this material.

Diagnostic records available at this laboratory indicate that some strains of chickens have a relatively high incidence of infectious synovitis in commercially reared flocks and that other strains have little or none. In the strains with a relatively high incidence of infection, the disease is recognized in only a portion of groups hatched. This suggests that if the disease is transmitted via the egg it occurs in only a small percentage of the progeny. Although some genetic differences between strains or breeds in susceptibility to infectious synovitis may occur, there has been no evidence presented to date indicating difficulty in artificially infecting any of the various strains and breeds used for experimental purposes.

MATERIALS AND METHODS

Breeding stock was obtained from a closed flock of Rhode Islands Reds free of pathogenic PPLO infection. During these trials the breeders were confined in isolated colony type houses and the progeny in batteries or colony houses.

Trial 1. Forty hens and 4 cocks 18 months of age were injected intravenously with 2.0 ml of a yolk-propagated agent of infectious synovitis. Eight 2-week-old chicks were similarly infected with 0.5 ml of the inoculum and all developed characteristic lesions within 9 days. No signs of infection were found in the breeding stock after 28 days and they were reinjected

with 1.0 ml of the agent. On the 2nd, 4th, and 6th day following the second injection, each bird was injected intramuscularly with 10 mg of cortisone acetate. Eggs were collected for incubation for 10 days following the first injection and between the 10th and 20th days following the second injection with the synovitis agent. A total of 449 eggs was incubated during these periods. Chicks that hatched were vaccinated with fowl pox during the 7th week of age and were necropsied at 10 weeks of age. The breeding stock was necropsied 16 weeks following initial injection.

Trial 2. A group of 35 hens and 4 cocks 18 months of age was injected intravenously with 3.0 ml of a yolk-propagated isolate of infectious synovitis proven to be infectious for 3-week-old chicks in 0.2 ml quantities. Blood and liver was collected from these test chicks on the 7th day postinfection when lesions were marked. The livers were ground with equal volume of whole blood and the total diluted 1:1 with nutrient broth. Each was injected intraperitoneally with 4.0 ml of this material. All birds were necropsied 8 weeks following the initial injection.

Trial 3. A group of 20 hens and 3 cockerels 6 months of age was injected intravenously with 3.0 ml of yolk material from the third embryo passage of a recent isolate of infectious synovitis. This material produced lesions in 4-week-old chicks given 0.5 ml intravenously. Eggs were saved for incubation from the 3rd to 60th day postinfection. After the 5th day of incubation, all eggs were candled daily. At least 3 blind passages were made in 6-day-old embryos by yolk or liver from pools of not more than 6 dead embryos. All chicks hatched were held in isolation until necropsy at 10 weeks of age. The breeding stock was necropsied 62 days postinjection.

RESULTS

No symptoms or lesions were found in any of the 44 mature birds used in trial 1. The chicks reared from this breeding stock also failed to show lesions at necropsy. No symptoms or lesions were observed in the injected birds in trial 2 except a sharp temporary drop in production following the intraperitoneal injection of the blood and liver suspension. At necropsy, firm orange or salmon colored exudate, considered

typical of infectious synovitis of considerable duration, was found in several of the major joints of 1 hen and 1 cock. The gonads of both birds appeared fully active.

In trial 3 a total of 30 pools of embryonic tissue representing 91 dead embryos was passed in the yolk sac of 6-day-old embryos to attempt to demonstrate the presence of the agent of infectious synovitis. The first isolation was made from a pool of yolk collected from 2 embryos dead on the 11th day of incubation. These embryos had developed from eggs collected between the 3rd and 10th day following injection of the breeding stock. Lesions were not observed in either embryo but characteristic lesions were seen in embryos on the first yolk passage of the yolk material. Yolk harvest from these embryos produced typical lesions of infectious synovitis in chicks injected intravenously. A second isolation was made from the yolk of a single embryo dead on the 12th day of incubation from eggs collected between the 11th and 29th day postinfection. Characteristic lesions of infectious synovitis were not observed until the 4th embryo passage of the material. Intravenous injection of yolk material from this passage produced characteristic lesions in chicks. A total of 212 chicks was reared in isolation to 10 weeks of age but did not show lesions of infectious synovitis at necropsy. One cockerel from the breeding stock was removed on the 23rd day and one hen on the 30th day postinjection in a moribund condition due to widespread lesions of infectious synovitis. At necropsy, 62 days postinjection, lesions of infectious synovitis were found in one cockerel and five hens. Three of these hens were in active egg production and had not shown signs of infection. The agent was reisolated only from the two moribund birds.

DISCUSSION

One of the major difficulties encountered in attempting to demonstate egg transmission of infectious synovitis under experimental conditions in this laboratory has been to induce infection in mature chickens. No such difficulty has been encountered in chickens between 28 and 104 days of age. This appears to indicate that an appreciable degree of resistance to infectious synovitis develops by the time of sexual maturity of the chicken. The higher incidence of infection secured in

trial 3 as compared to trials 1, and 2 may have been due to greater pathogenicity of the agent used or to greater susceptibility of 6-month-old birds as compared to 18-month-old birds. Although large volumes of material containing the agent were given in all trials, the dosage was probably different in each trial.

The two recorded instances of isolation of the agent of infectious synovitis from dead embryos indicates that transmission of the disease via the egg may occur in the field. The second isolation from an egg laid between 11th and 29th days postinfection may be more significant than the first isolation from an egg which may have been laid as early as 48 hours following intravenous injection of the agent. It is not known if the eggs were infected by transovarian means or by penetration of the agent through the shell at the time of being laid or at a later time.

More investigation is necessary to determine the role of egg transmission in the epizootiology of infectious synovitis.

SUMMARY

Considerable difficulty was encountered in experimentally infecting mature chickens with infectious synovitis. This appears to be a result of age resistance, as chicks are quite readily infected. The agent producing this disease was isolated on two occasions from the yolk sacs of dead embryos from eggs laid by birds exposed to the agent. This finding supports previous evidence that egg transmission of infectious synovitis may occur.

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INFECTIOUS SYNOVITIS I. CLINICAL AND PATHOLOGICAL MANIFESTATIONS*

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EARLY reports that infectious synovitis was a newly recognized disease of chickens were published in 1954.[1,6,8] Later the disease was identified in turkeys.[7] Recent diagnostic reports from various states indicate that infectious synovitis of chickens and turkeys remains a problem in several sections of the United States.[5] Aids in the identification of the disease therefore are of increasing concern to the diagnostician. History, signs, gross and microscopic lesions of birds in affected flocks in most instances have given the diagnostician sufficient information for a tentative diagnosis of infectious synovitis. For confirmation, he has resorted to isolation techniques with chicken embryos and reproduction of the disease in young chickens.

Signs and lesions in natural infections. In Massachusetts, infectious synovitis has been diagnosed more frequently in chickens from 4 to 16 weeks old, and in turkeys 10 to 24 weeks old than at other ages. The disease has not been as common or severe in turkey flocks as in chicken flocks. Birds with this disease have been submitted to the diagnostic laboratory most frequently because the poultryman had noticed them to be lame or reluctant to walk. Morbidity during the early phase of the disease was not always apparent but

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severely affected birds usually appeared weak and depressed. A green diarrhea was a frequent sign. Occasionally, only one infected bird was submitted from a flock where no other birds were apparently affected. Gross enlargements in the areas of the hock, wing joints, foot pad and sternal bursa were seen. Although the mortality rate usually has been low (about 3 percent), the morbidity has been as high as 50 percent. In unmedicated, infected flocks, the disease has progressed and lasted for several months so that affected birds became thin and dehydrated.

At necropsy, the lesions of infectious synovitis depended on the severity and duration of the infection. During the early stages of the disease, the liver was enlarged and often appeared either mottled, or green, or both. The spleen was enlarged and speckled with grayish-white foci (figure 3). In more advanced cases, the synovial membranes, particularly around the sternal bursa (figure 1), hocks (figure 2), phalanges and wing joints, were thickened and contained a yellow to yellow-orange, creamy, viscid exudate. When the extremities were involved, lesions have been unilateral or bilateral. The bone marrow at times was pale. During the latter stages of the infection, the appearance of the spleen and liver returned to normal whereas the synovial exudate became coagulated, firm, and salmon-colored. Similar material occasionally was seen at this time in the subcutaneous tissues around the posteroventral aspects of the cervical region.

Microscopic lesions of the liver have been a valuable aid in the diagnosis of infectious synovitis. Focal proliferation with resulting aggregations of reticular cells were seen in the periportal and sinusoidal areas (figure 5). The cells of the aggregates had large distinct vesicular nuclei with hyperchromatic nucleoli. When liver sections were stained with Gridley's reticulum stain, the cells were seen interspersed among many reticular fibers (figure 6). The sinusoids were dilated and the parenchymal cells were atrophied. There was proliferation of bile duct epithelium.

Isolations from natural infections. Synovial exudate from affected birds was withdrawn aseptically with a 16 gauge

Fig. 1. Photograph of the sternal bursa of an experimentally infected chicken. Note gelatinous to cheesy exudate.
Fig. 2. Photograph of exposed hock joints. Synovial sacs around right joint affected with infectious synovitis; left, control.

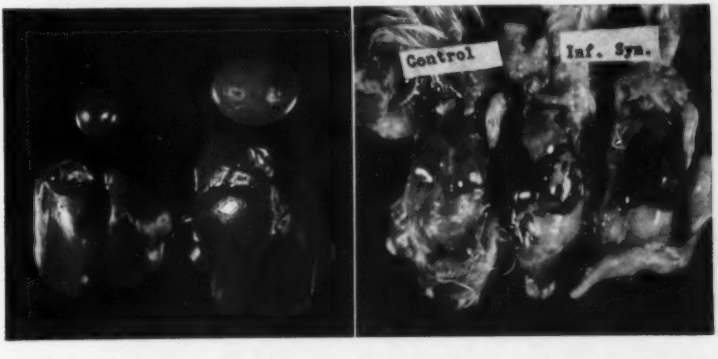

Fig. 3. Photograph of enlarged liver and spleen (right) from experimentally infected chicken 8 days post-inoculation; left control.
Fig. 4. Photograph of affected livers of chicken embryos with infectious synovitis. Embryo to left is normal control.

needle and syringe or swabbed with sterile cotton swabs. Several drops of exudate or the swabs were placed in 1.5 ml of sterile broth. Occasionally, several drops of blood were withdrawn aseptically during the early stage of infection and were similarly suspended. The suspensions were inoculated into the yolk sacs of 6-day-old chicken embryos using 0.2 ml of the inoculum per egg. If the infectious synovitis agent (ISA) grew, the embryo mortalities were erratic and scattered throughout the period of incubation. Surviving embryos were sacrificed at 20 days of incubation and examined for lesions. Affected livers were enlarged and reddened, and occasionally had focal, greenish-yellow discolorations (figure 4). The spleens were enlarged and reddened. The pericardial sac was sometimes distended with straw-colored fluid which at times contained a grayish-white flocculant material. The cardiac muscle was pale. Microscopic examination of embryo livers infected with the ISA had distinctive focal accumulations of reticular cells in perivascular and sinusoidal areas particularly during the last trimester of incubation (figure 7). This observation has been a valuable criterion in the identification of the ISA.

At the time of embryo inoculation and at the time when death or lesions occurred, parallel cultures of the inoculum and yolk harvest were made on chicken infusion agar, blood agar, PPLO broth and agar (Difco). Evidence of bacterial growth on, or in, any of these media when inoculated with the ISA could not be demonstrated.

EXPERIMENTAL PROCEDURES

Tissue and blood examinations were made on a group of 32 Rhode Island Red chicks derived from a closed flock known to be free of chronic respiratory disease. The chicks were fed a commercial ration and were reared in isolation. At 4 weeks of age, half of the group was inoculated intravenously with approximately five chick-infective-doses of the ISA (8585) and the other half was maintained as control birds. Five days post inoculation, the affected birds appeared depressed and listless and had green diarrhea. Hemagglutination inhibition,

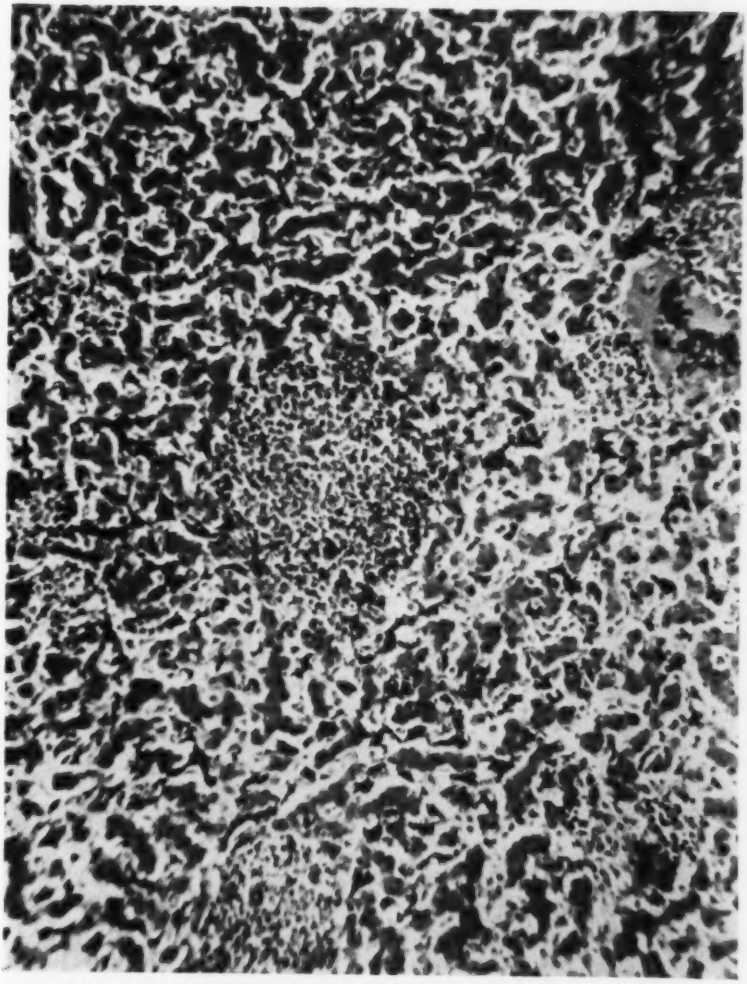

Fig. 5. Photomicrograph of a liver section with focal aggregates of reticular cells from a naturally infected chicken x200.

serum neutralization, and the rapid serum plate tests were negative for Newcastle disease, infectious bronchitis, and chronic respiratory disease respectively throughout the experiment.

Blood and tissues were collected from two birds of both of the infected and non-infected groups as they were sacrificed on the 2nd, 5th, 7th, 9th, 12th, 14th, 16th and 19th days post inoculation. Erythrocyte and leukocyte counts were made according to the technique outlined by Natt and Herrick.[4] Differential counts were made on heterophils, lymphocytes, eosinophils, monocytes, basophils, and thrombocytes. Hemoglobin readings were taken with a Spencer hemoglobinometer. The rest of the blood examinations were made according to standard procedures as described by Coffin.[3] Soft tissues were fixed in 10 percent buffered formalin; hard tissues were fixed in a mixture consisting of 85 parts picric acid, 10 parts of 40 percent formaldehyde, and 5 parts of formic acid. Tissue sections were cut 6 microns thick. All tissues were stained with hematoxylin and eosin and where noted, with Gridley's reticulum stain.

Host susceptibility trials were conducted on 5-week-old pheasants, and 4-week-old geese and ducks. Approximately five chick-infective-doses of the ISA were given via the vein to pheasants and geese and the tendon sheath to ducks.

RESULTS

Hematology. The results of examinations of blood from birds with infectious synovitis from one trial are found in table 1. Replicate trials have produced similar results. The thrombocyte, heterophil, monocyte, and total leukocyte counts and sedimentation rate of infected birds were greater than those in the control group; on the other hand, the hematocrit, hemoglobin content, erythrocyte and lymphocyte (relative but not necessarily absolute) counts were less. The blood disturbances were most pronounced between the 5th and 16th days post inoculation.

Pathology. Vascular endothelial thickening and adventitial proliferation were seen in the cerebrum, cerebellum, and

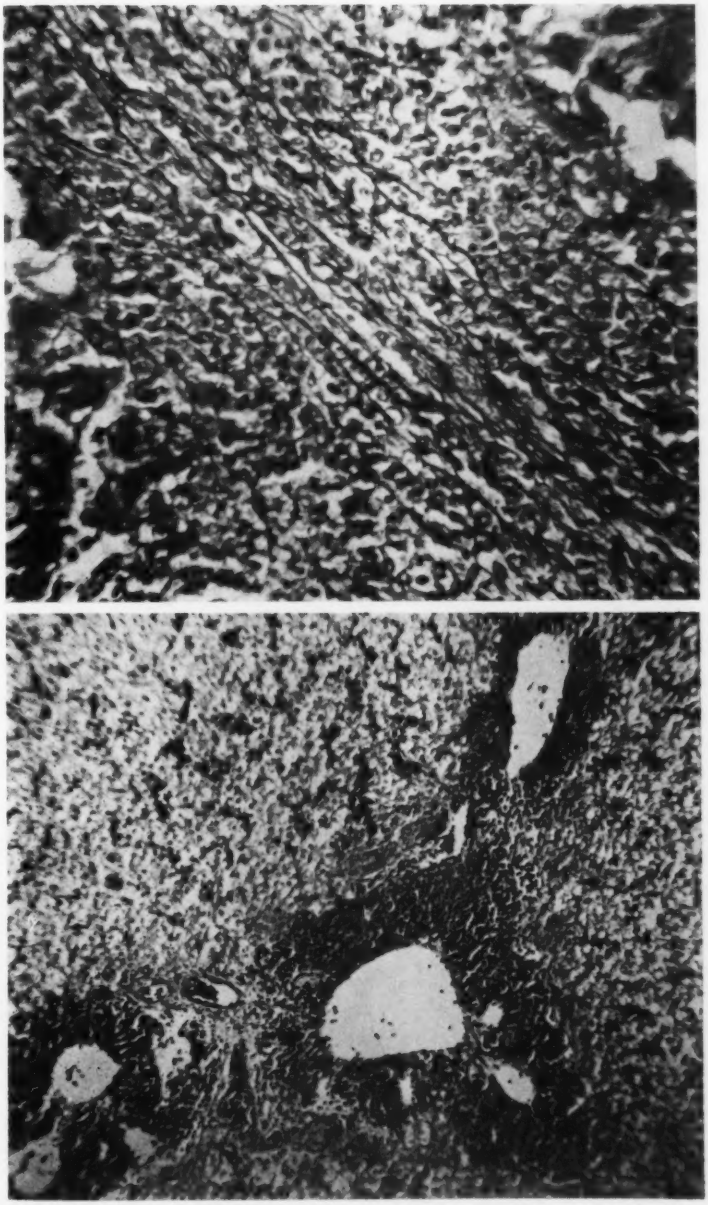

Fig. 6. (top) Photomicrograph of liver section with focal aggregates of reticular cells interspersed among reticular fibers from a naturally infected chicken. Gridley's reticulum stain x240.

Fig. 7. (bottom) Photomicrograph of a liver section from a chicken embryo affected with infectious synovitis. Note accumulation of reticular cells x240.

TABLE I

Data from one trial† on blood changes in 4-week-old chickens with infectious synovitis

		Days Post Inoculation								
		0	2	5	7	9	12	14	16	19
Hematocrit, percent	I*	52	27.5	25	26	26.5	21	26.0	27	28
	C		32	27	30	31.5	30	29.0	31	29.5
Hemoglobin ,Gm/100 ml	I	11.2	10.9	9.2	10.0	10.5	9.1	10.5	10.6	9.7
	C		11.1	8.8	13.0	11.0	12.0	12.6	12.7	11.7
Erythrocytes (millions/cu. ml)	I	2.5	2.8	2.3	2.0	2.2	1.9	2.1	2.1	2.8
	C		3.2	2.6	2.6	2.6	2.2	2.0	2.2	2.7
Thrombocytes (thousands/cu. ml)	I	18	29.2	59.6	119.5	93.5	62.1	103.3	150.4	73
	C		16.2	37.8	34.5	41	27.9	48.1	46.2	64.5
Leukocytes (thousands/cu. ml)	I	29	21	71	146	117	79.5	51	102	80.5
	C		17.7	34.5	55	45	19.5	33.5	30	41
Differential counts (percent)										
Heterophils	I	39	28	56	26	23.2	44	54.5	52	51.5
	C		30.5	45.5	30.5	31.5	27	19	25	20
Eosinophils	I	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0
Basophils	I	1	2	2.5	0.5	1.0	1	1.0	1.5	1
	C		1.5	2.5	3.5	4.0	4	2.5	1.5	4.5
Lymphocytes	I	57	60.5	32	60.5	73	48	38.5	39.5	43
	C		64.5	48	61.5	64.5	69	79.5	71.5	75
Monocytes	I	3	9.5	10	13	3.2	7	5.0	7	5
	C		3.5	3.5	3.5	2.0	2	4.0	2.0	2
Sedimentation Rate, mm/hr	I	1.5	1.75	1.5	0.5	0.7	1,2	1.2	0.75	.75
	C		1.75	1.0	1.0	1.0	1.0	1.0	·.75	1.25

† Comparable results obtained in replicate trials.
* I = infected, C = control.

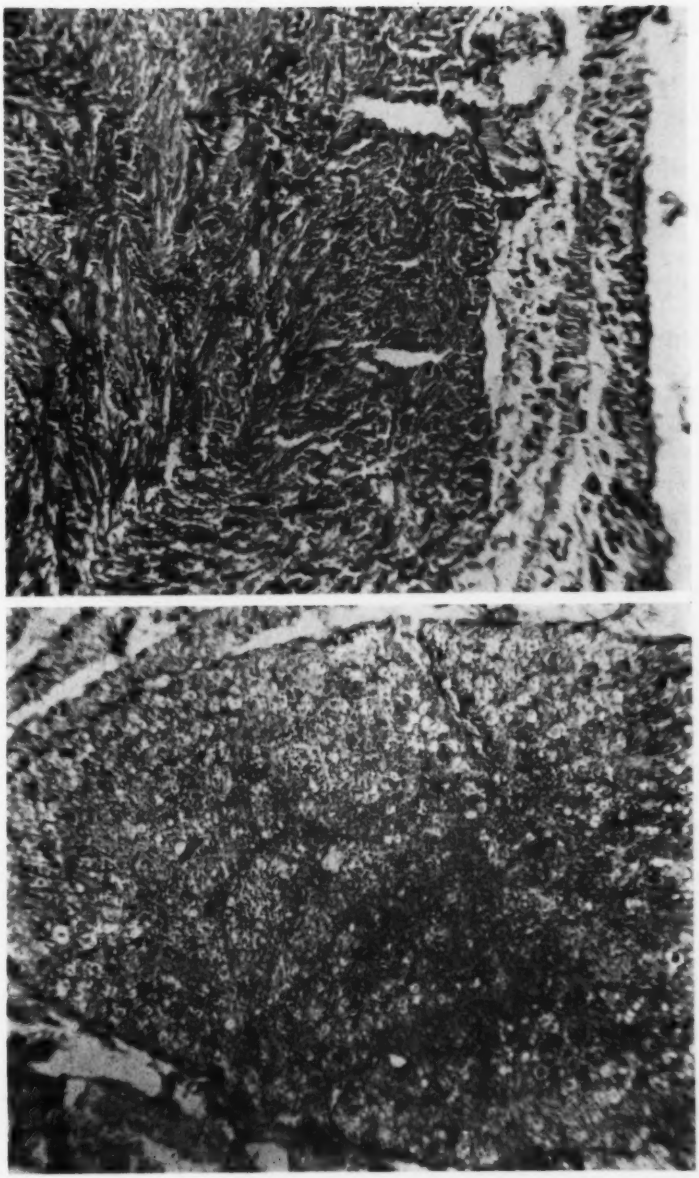

Fig. 8. (top) Photomicrograph of a heart section of a 5-week-old chicken 9 days post inoculation. Epicardium is edematous and the myocardium is infiltrated by mononuclear cells x240.

Fig. 9. (bottom) Photomicrograph of a section of thymus with lymphoid cell degeneration from a 6-week-old chicken 14 days post inoculation x120.

optic lobe of chicks with experimental infectious synovitis. There was degeneration of some of the Purkinje cells. Two of sixteen infected chicks had cerebellar lesions similar to those of encephalomalacia. Brain sections from all control birds appeared normal.

Gross enlargement and mottling of the liver were seen from about the 5th to the 16th day post inoculation. Perivascular, periportal and interparenchymal cellular hyperplasia was seen microscopically within 5 days post inoculation. Evidence of mitotic activity was seen in some of these cells. Masses of reticular fibers were present among these cells. Because of their morphology, location, mitotic status, and association with reticular fibers, these cells were interpreted to be reticular cells of the reticuloendothelial system. Microscopic lesions of the liver were not as pronounced at 16 and 19 days post inoculation as during the earlier phase of the infection.

There was cellular hyperplasia along the interlobular septa of the lungs and the connective tissue framework of the gizzard and heart. These cells were morphologically similar to those described in the liver. Less frequently, focal mononuclear infiltration and necrosis of the myocardium and a fibrinous inflammation of the epicardium were seen (figure 8).

Gross enlargement and paleness of the spleen were present from about the 5th to the 16th days post inoculation. Sinusoidal areas appeared decreased as a result of reticular cell or lymphocytic hyperplasia, or both. Atrophy of the thymus and bursa of Fabricius resulted from a lymphoid cell degeneration in the medulla and cortex (figures 9, 10).

There was a predominantly heterophilic inflammation involving the sternal bursa and the adjacent subcutaneous tissue.

Within 5 days after inoculation, there was a granulocytic hyperplasia in the bone marrow which was gray on gross appearance (figure 11). Synovial sacs and membranes around joints and tendons were thickened and contained inflammatory exudate (figure 12). Articular cartilage, however, had little to no evidence of inflammation.

Host susceptibility. Five of seven 5-week-old pheasants

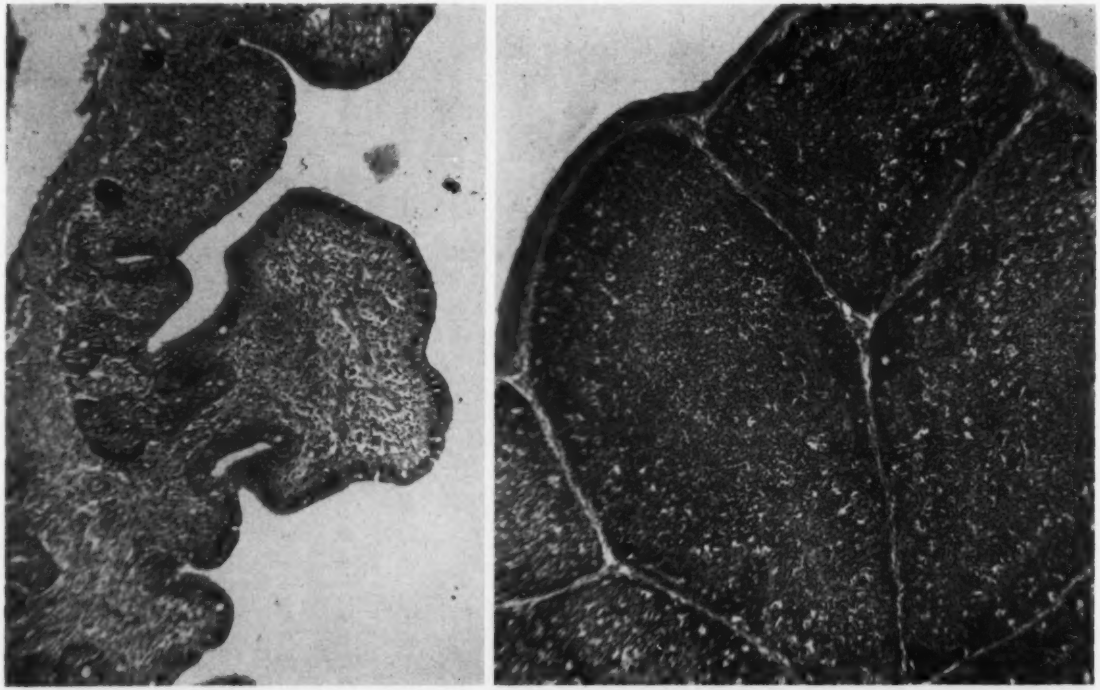

Fig. 10. Photomicrograph (left) of section of bursa of Fabricius with lymphoid cell degeneration and atrophy of lobules from a 6-week-old chicken 14 days post inoculation. Photomicrograph (right) of bursa of Fabricius section from control bird x120.

were infected with the ISA in one trial when the inoculum was given by the intravenous route. The agent was recovered from the synovial exudate when injected into the yolk sac of 7-day-old chicken embryos. One of four 4-week-old geese developed a synovitis when similarly inoculated but attempts to re-isolate the agent were not successful. Attempts to infect ducks via the tendon sheath were not successful.

DISCUSSION

Though infectious synovitis is a disease of chickens and turkeys only recently recognized, its economic importance is being felt in many parts of the United States. It is a debilitating disease of high morbidity, particularly in young birds. Affected birds are not marketable.

The signs of infectious synovitis may be confused with neurolymphomatosis, ruptured gastrocnemius tendons, staphylococcal arthritis, perosis, rickets or coccidiosis. However, the etiologic agent as well as certain lesions are distinct. When a group of birds with infectious synovitis was submitted to the diagnostic laboratory, it was usual to find them in various stages of infection. Experimentally produced liver and spleen lesions have been seen for limited times—5 to 15 days post-infection. However, synovial lesions have lasted much longer. Therefore, it was not unusual to find synovial lesions without apparent liver and spleen changes in natural infections. The presence of yellow to yellow-orange, creamy, synovial exudate was the most striking lesion of infectious synovitis. Most of the isolations made at this station have been made from this material. The etiologic agent was easily grown and was pathogenic when placed in the yolk-sacs of 6-day-old embryonating chicken eggs.

There have been no reports to date indicating that the ISA can be grown on artificial media. It has been reported that the size of the agent is in the classification of a small bacterium or large particle virus.[2] More work is needed to classify this agent properly.

Little is known about the epizootiology of infectious synovitis. It is not a highly contagious disease. Egg trans-

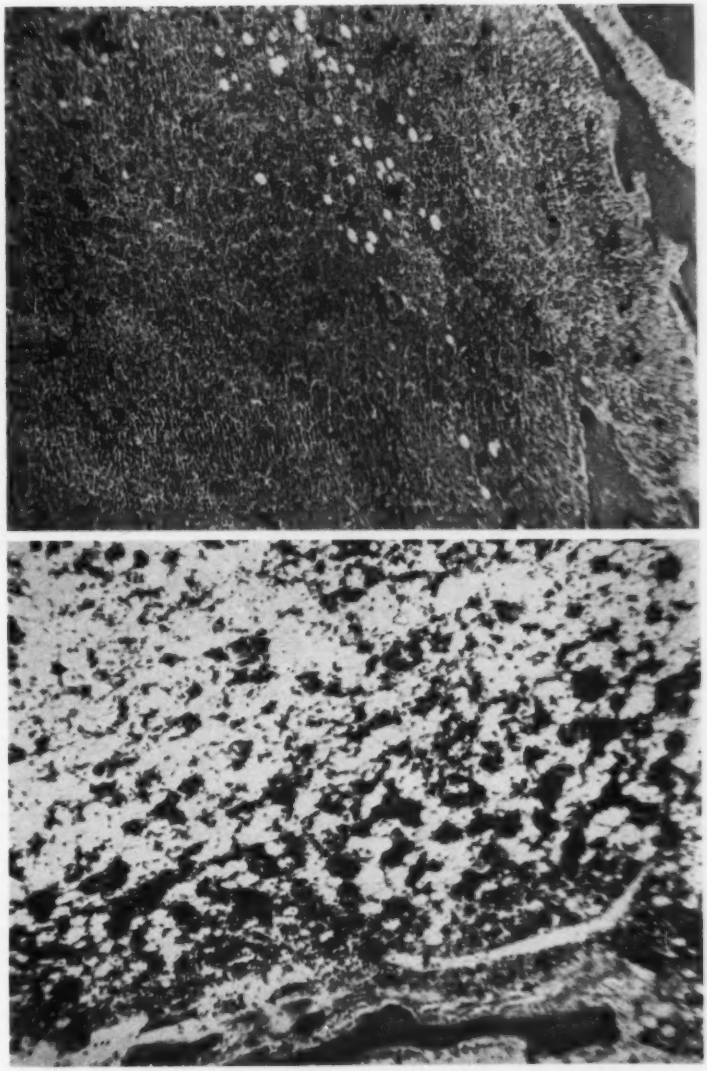

Fig. 11. Photomicrograph (top) of section of femur with myelocytic hyperplasia from a 5-week-old chicken 9 days post inoculation. Photomicrograph (bottom) of a section of femur from control bird x120.

Fig. 12. Photomicrograph of section through the tibiometatarsal joint from a 6-week-old chicken. Note the thickened synovial sac containing inflammatory cells and the lack of inflammation of the articular surface of bones x50.

mission trials are still in progress at this station to fully explore this possibility. Until more is known, sound recommendations on the disposition of infected breeders and replacement stock will be lacking because treatment has been of little value.

ACKNOWLEDGMENT

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SUMMARY

Infectious synovitis is an infectious disease of chickens and turkeys characterized primarily by involvement of synovial and hepatic tissues.

Signs of the disease are lameness, reluctance to walk, depression, and green diarrhea. Mortality is low but morbidity has been up to 50 percent.

Most pronounced lesions of affected birds are a heterophilic inflammation and exudation of the synovial membranes, reticulocytosis of the liver, pericarditis, and myelocytic hyperplasia of the bone marrow.

Isolations from tissues of infected birds have been made in 6-day-old chicken embryos which, when infected, have a characteristic reticulocytosis of the liver.

Inoculation of the infectious synovitis agent into young chickens has reproduced the disease as it is seen in natural cases.

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INFECTIOUS SYNOVITIS: II. DRUG PROPHYLAXIS AND THERAPY*

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THE principal object of the trials reported here was to investigate various antibiotics and drugs which might prove practical for treating or preventing infectious synovitis of chickens and turkeys. Since the initial reports of infectious synovitis in chickens in 1954[5,18] and in turkeys in 1955[16] a number of investigators have reported on the sensitivity of the causal agent to various antibiotics and other drugs. Most of these sensitivity trials have been carried out by the use of chicken embryos or growing chickens or both. Penicillin, bacitracin para-aminobenzoic acid, several sulfonamides and erythromycin have been reported to show little or no activity against the agent.[3,4,6,7,10,11,12,19,20] Chloramphenicol has been reported to show appreciable activity against the agent.[4] The causal agent of infectious synovitis has been reported to be susceptible to the tetracyclines, furazolidone and the streptomycins.[3,4,6,7,10,11,12,18,19,21] Apparently because of lack of absorption by the gut, the streptomycins do not show actively against infectious synovitis in birds unless administered parenterally.[7,10,11]

Dihydrostreptomycin has been reported to be relatively effective prohylactically if administered parenterally at the time of infection; little benefit was secured if administration was delayed until signs of the disease were seen.[15]

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The tetracyclines and furazolidone have been shown to possess considerable activity against the agent and have shown the most promise for control of the disease by medication via the feed. Reports of critical comparisons of the effectiveness of these compounds when administered in the feed in preventing development of lesions in experimentally infected birds have shown the following descending order of effectiveness; chlortetracycline, oxytetracycline, tetracycline and furazolidone.[7,8,11,12,13,21] Although appropriate levels of these compounds are effective in preventing appearance of lesions during medication they have not prevented the development of lesions after terminating medication.[1,8,11] Slight therapeutic activity has been demonstrated by these compounds.[1,14] Considerable variability in the levels of these drugs necessary in feeds to prevent infectious synovitis has been found by different investigators.[2,7,8,9,10] Only two reports have appeared regarding prophylaxis of infectious synovitis in turkeys.[2,11] Neither of these reports indicates an essential difference between the prophylactic activity of a number of compounds in turkeys as compared to chickens. Cover *et al.*[3] reported one isolate of the agent which showed appreciably more resistance than four other isolates to furazolidone, streptomycin, oxytetracycline, and tetracycline when tested *in ovo*.

MATERIALS AND METHODS

The isolates used for testing drug sensitivity were secured from field infections of several breeds and strain crosses of chickens and one from a turkey flock. No selection of these strains was made on a basis of drug sensitivity. Most attention was given to comparing the relative protective activity of chlortetracycline (CTC), oxytetracycline (OTC) and furazolidone. Isolates of the agent were propagated in the yolk sac of 7-day-old chicken embryos; yolk harvest from dead embryos was used for infecting trial birds. All infections for drug trials were produced by intravenous (IV) injection of chickens or turkeys between 28 and 35 days of age. Previous work indicated that this route produced a more consistent infection rate and a more uniform incubation period than infec-

tion by intramuscular, intratracheal or tendon sheath introduction of the agent. No infection was produced by the oral route. Infected and non-infected birds were held in the same room but in separate wire-floored batteries. The chicks were progeny of PPLO-free breeders and were maintained free of clinical evidence of infection with CRD or other contagious diseases. Representative birds from some trials were tested by standard serological methods and demonstrated to be susceptible to Newcastle disease and infectious bronchitis and unaffected by PPLO at the end of the trial.

The drugs which were administered in the feed were mixed with commercial growing mash one or two days prior to starting the trial and at 10 to 14 day intervals during the trial when necessary. Commercial water-soluble forms of OTC and CTC were used for feed mixtures of these compounds. A commercial 11 percent pre-mix of furazolidone was used. Several analyses of trial mixtures of this compound indicated a final concentration in the mixture of 9 to 10 percent under the calculated level; for this reason, all furazolidone levels tested were mixed with a calculated 10 percent surplus of the drug. The other drugs were mixed without overrun but were not assayed. All feed medication levels are indicated at grams per ton of the total ration and were fed ad libitum (e.g. 100 grams per ton equals approximately 0.011 percent or 91 ppm.). Feed medication was started at the time of infection and continued until the end of a trial.

At the termination of a trial all birds were necropsied. Major joints and synovial tissues were examined for the presence or absence of lesions; in some trials, this was used as the sole criterion for comparing drug activity. In other trials, a scoring system was used to assist in comparing drug activity. This system was based on assigning a value of 1 to 4 depending upon the extent of lesions in each of the following synovial tissue areas: humero-radial, radio carpal, mandibular, femoro-tibial, tibio-metacarpal, and metacarpo-phaangeal joints and the sternal bursa. This system allowed a maximum possible score of 52 per bird if severe lesions occurred in each of these areas.

TABLE I

Relationship of age of chicks and dose of the infectious synovitis agent to development of lesions

Group	Age days	Weight lbs.	Dosage	Lesions	Group Score
1	23	1/2	0.1 ml	5/10	102
2	23	1/2	0.2 ml	10/10	259
3	23	1/2	0.3 ml	10/10	223
4	37	1	0.2 ml	8/10	166
5	37	1	0.4 ml	10/10	173
6	58	1 3/4-2 1/2	0.2 ml	7/10	83
7	58	1 3/4-2 1/2	0.4 ml per lb.	10/10	95
8	104	2 1/2-4 3/4	0.2 ml	10/10	152
9	104	2 1/2-4 3/4	0.4 ml per lb.	10/10	108

Isolate #9895 necropsied 14 days postinjection.

TABLE II

Relationship of dosage of the infectious synovitis agent to resulting infection and drug prophylaxis

Group	Volume of agent per bird* in ml	Chlortetracycline medication	Incubation period	Lesions†	Group Score
1	0.025	75 gm/ton		0/7	0
2	0.025	none		0/7	0
3	0.05	75 gm/ton		0/7	0
4	0.05	none		0/7	0
5	0.1	75 gm/ton		0/7	0
6	0.1	none	8 days	6/7	70
7	0.2	75 gm/ton		0/6	0
8	0.2	none	6 days	6/6	145
9	0.4	75 gm/ton		0/6	0
10	0.4	none	5 days	6/6	195
11	0.8	75 gm/ton		0/6	0
12	0.8	none	3 days	6/6	171
13	1.6	75 gm/ton		0/6	0
14	1.6	none	3 days	6/6	157

* All injected IV with undiluted pooled harvest of isolate 9589.
† All birds necropsied 14 days postinjection.

RESULTS

The drug prohylaxis trials summarized in tables 2 through 5 are representative of a total of 17 trials involving a total of 560 turkeys and 1210 chickens. Seven isolates were tested for drug sensitivity; trial results of two of the isolates are not shown as the drug sensitivity range of these was within the limits demonstrated by those tabulated.

The trial summarized in table 1 was designed to test a

possible relationship between the size of the infecting dose and the severity of lesions produced as well as age susceptibility. Although the number of birds used was relatively small, the results indicate that in this instance the administration of 0.2 ml IV of yolk-propagated agent was adequate to infect nearly all birds. The experiment summarized in table 2 tends to support this evidence that a relatively large volume of yolk-propagated agent is necessary to produce consistent infection. Other investigators likewise have used approximately this volume of material to infect chickens.[2,6,20] In this trial, birds 23, 37, 58 and 104 days of age were readily infected. Increasing the infecting dosage beyond 0.2 ml did not appear to influence the severity of lesions within the age group although the clinical signs were observed several days earlier in each age group receiving the larger dosage of approximately 0.4 ml per pound of body weight. It was also noted that the onset of signs was less sharply defined as the birds advanced in age and that the signs were less severe.

The trial summarized in table 2 was designed to determine if the volume of infective material influenced the course of the disease or the apparent efficacy of a prophylactic drug. Separate groups of 5-week-old chicks were injected starting with 0.025 ml of yolk harvest and increased to 1.6 ml of yolk material by serial doubling of dosage. Duplicate groups at the various dosage levels were fed chlortetracycline at a 75 gram per ton level throughout the trial. The 0.025 and 0.05 volumes were made up by 1:10 dilutions with nutrient broth to secure a more readily measured volume. A separate trial not included in this report indicated that such broth dilution had little or no effect on the agent. No infection was secured until a dosage of 0.1 ml was used. At this level, 6 of 7 birds were affected and all birds were infected at larger dosages. At both the 0.8 and 1.6 ml dosage levels, abnormal droppings were noted by the 3rd day postinjection and the incubation period progressively increased to 8 days at the 0.1 ml dosage level. The group lesion score was appreciably lower at the 0.1 ml dosage level than at higher dosage. This may be chiefly a result of insufficient lapse of time following onset of symptoms be-

cause of the comparatively longer incubation period. Previous trials summarized in table 3 indicated that 75 grams per ton of chlortetracycline was approximately the minimal amount necessary to completely suppress lesions produced by isolate 9589 in chicks in this age range. In this trial, similar complete suppression was secured at all dosage levels.

Table 3 summarizes three separate trials with one isolate. Comparison of the separate trials demonstrates that results are reasonably reproducible from trial to trial. The three drugs, OTC, CTC, and furazolidone induce approximately the same relative protection in turkeys as compared to chickens. Feed levels of 50 grams per ton of CTC either completely prevented the development of lesions or reduced the severity and extent of lesions to a very low order. Comparable control was secured by a 100 gram per ton level of OTC. Although some reduction in severity and extent of lesions was secured by a 200 gram per ton level of furazolidone, it was not as great as that secured by either a 50 gram per ton level of CTC or a 100 gram per ton level of OTC.

The four trials summarized in table 4 are presented to demonstrate variable drug sensitivity among several isolates of the agent. Isolate C442 in table 4 trial I, produced lesions in all birds fed 400 grams per ton furazolidone; in contrast, 200 grams per ton furazolidone prevented the development of lesions in birds infected with isolate #9762 shown in table 4 trial 4. With the latter isolate, complete protection was secured by 50 grams per ton CTC. In contrast, isolate #8585 required a 200 gram per ton level of CTC to prevent development of lesions since 150 grams per ton was inadequate.

Trials 1 and 2 also demonstrate the prophylactic activity of cathomycin* and erythromycin. An 800 gram per ton level of cathomycin decreased the extent of lesions but did not completely prevent them. A separate test with chickens with a different isolate produced comparable results. Erythromycin at

* Cathomycin, streptonivicin and cardelmycin, although isolated from different sources, are the same antibiotic.[17]

TABLE III

A comparsion of separate trials in chickens and turkeys testing the prophylactic activity of several drugs against infectious synovitis

Species	Age	Medication			
		Drug	Level	Lesions	Group Score
			Trial I		
Chickens	30 days	Positive controls		10/10	190
		Oxytetracycline	50 gm/ton	1/10	4
		"	100 gm/ton	3/10	3
		"	150 gm/ton	0/10	0
		Chlortetracycline	50 gm/ton	0/10	0
		"	100 gm/ton	0/10	0
		"	150 gm/ton	0/10	0
		Furazolidone	50 gm/ton	10/10	188
		"	100 gm/ton	9/10	119
		"	150 gm/ton	10/10	69
		"	200 gm/ton	10/10	53
			Trial II		
Turkeys	35 days	Positive Controls		10/10	96
		Oxytetracycline	50 gm/ton	10/10	36
		"	100 gm/ton	8/10	19
		"	150 gm/ton	0/10	0
		"	200 gm/ton	0/10	0
		Chlortetracycline	50 gm/ton	1/10	1
		"	100 gm/ton	0/10	0
		"	150 gm/ton	0/10	0
		"	200 gm/ton	0/10	0
		Furazolidone	150 gm/ton	10/10	136
		"	200 gm/ton	10/10	102

Trial III

Turkeys	34 days	Positive Controls		8/8	119
		Oxytetracycline	50 gm/ton	7/8	47
		"	200 gm/ton	1/8	2
		Chlortetracycline	50 gm/ton	6/8	22
		"	200 gm/ton	0/8	0
		Furazolidone	50 gm/ton	7/8	91
		"	100 gm/ton	8/8	69
		"	150 gm/ton	8/8	80
		"	200 gm/ton	8/8	49
Chickens	31 days	Positive Controls		7/7	188
		Furazolidone	50 gm/ton	9/9	183
		"	100 gm/ton	7/9	115
		"	150 gm/ton	8/9	81
		"	200 gm/ton	3/9	31

Injected with 0.3 ml of isolate #9589. Medication started at time of injection and continued to necropsy. Negative control groups in all trials remained negative.

TABLE IV

Prophylactic effect of several drugs against four isolates of infectious synovitis in chickens and turkeys

Species	Age	Drug Medication	Level	Lesions	Group Score
		Trial I	Isolate #C442		
Turkeys	28 days	Positive controls		10/10	Not scored
		Furazolidone	200 gm/ton	10/10	" "
		"	300 gm/ton	0/10	" "
		"	400 gm/ton	10/10	" "
		Chlortetracycline	200 gm/ton	0/10	" "
		Cathomycin	200 gm/ton	8/10	" "
		"	300 gm/ton	5/10	" "
		"	400 gm/ton	5/10	" "
		"	800 gm/ton	6/10	" "
		Trial II	Isolate #9895		
Chickens	35 days	Positive Controls		9/10	163
		Erythromycin	200 gm/ton	8/10	107
		"	400 gm/ton	9/10	79
		"	800 gm/ton	10/10	133
		"	200 mg at infection*	6/10	56
		Oxytetracycline	100 gm/ton	0/10	0
		Chlortetracycline	100 gm/ton	0/10	0
		Trial III	Isolate #8585		
Chickens	28 days	Positive controls		10/10	Not scored
		Chlortetracycline	50 gm/ton	8/10	" "
		"	100 gm/ton	7/10	" "
		"	150 gm/ton	4/10	" "
		"	200 gm/ton	0/10	" "
		Furazolidone	50 gm/ton	10/10	" "
		"	100 gm/ton	10/10	" "
		"	150 gm/ton	6/10	" "
		"	200 gm/ton	6/10	" "

		Trial IV	Isolate #9762		
Chickens	28 days	Positive controls	0.3 ml	10/10	134
		Oxytetracycline	50 gm/ton	1/10	23
		"	100 gm/ton	1/10	1
		"	200 gm/ton	0/10	0
		Chlortetracycline	50 gm/ton	0/10	0
		"	100 gm/ton	0/10	0
		"	200 gm/ton	0/10	0
		Furazolidone	50 gm/ton	5/10	63
		"	100 gm/ton	5/10	33
		"	200 gm/ton	0/10	0

Injected with 0.3 ml of the various isolates. Medication started at time of injection and continued to necropsy except one group injected subcutaneously with erythromycin*. Non-infected controls in each trial were negative at necropsy.

an 800 gram per ton level demonstrated little or no prophylactic activity; a separate trial with a different isolate produced comparable results.

The trial summarized in table 5 indicates the activity of dihydrostreptomycin administered subcutaneously at various dosages and periods following injection. A dosage of 125 mg afforded complete protection if administered either at the time of injection or 48 hours postinjection. Partial protection was afforded by 50, 125 and 250 mg at the 4th day postinjection and by 125 mg at the 6th day postinjection. In this trial, definite clinical signs were not observed until the 8th day postinjection and 125 mg was without apparent benefit when administered at that time.

TABLE V

Prophylaxis and treatment with dihydrostreptomycin-sulfate subcutaneously in 33-day-old chicks

	Medication		Lesions		
Group	Time	Dosage	Necropsy	No. affected	Score
1	Negative Control		22 days PI	0/10	0
2	Positive Control		14 days PI	9/10	178
3	at infection	125 mg	14 days PI	0/10	0
4	2 days PI	125 mg	22 days PI	0/10	0
5	4 days PI	50 mg	14 days PI	4/10	16*
6	4 days PI	125 mg	22 days PI	6/10	95
7	4 days PI	250 mg	22 days PI	6/10	58
8	6 days PI	125 mg	14 days PI	5/10	73
9	8 days PI†	125 mg	14 days PI	10/10	194

Injected at 33 days (average weight 0.7 lbs.) of age IV with 0.3 ml isolate #9895.

* 7/10 show very marked enlargement of liver and spleen indicating early acute infection.

† Distinct lameness and enlargement of joints at time of treatment.

PI = post inoculation.

DISCUSSION

A series of laboratory trials comparing the prophylactic effectiveness in chickens and turkeys of a variety of drugs against several isolates of infectious synovitis are reported. Although both species could be infected by a variety of parenteral routes and by the respiratory tract, the intravenous route produced the most consistent infection pattern and

appeared to be the route of choice for medication trials. By this route, a relatively large dose (0.1 ml or more) of yolk-propagated agent was necessary to produce consistent infection. A larger dosage shortened the incubation period but did not otherwise affect the course of the disease. The size of the infecting dose appeared to have little or no influence on the efficacy of a prophylactic drug. The shortest incubation period observed in this laboratory has been 3 days; the longest reported in this series of trials, in which relatively large dosages were used, was 8 days. Considerably longer incubation periods have been observed with smaller dosages which produced a low incidence of infection.

The scoring system used was of some value in assessing drug prophylactic activity. Rather large differences in scores appeared necessary to indicate significance. It was also necessary to make comparative scoring during comparable stages of the disease. Mortality and signs were poor criteria of the extent of infection; lesions were often found in birds that failed to show signs of infection. Weight gain differences were not satisfactory to measure drug protection under these test conditions because slight lesions often did not appreciably affect growth. There was, however, a general improvement in growth as effective drug levels were approached and when complete protection was secured, growth rates were normal. The most satisfactory index of drug activity was the presence or absence of lesions at necropsy.

No striking difference was found in ability to infect chickens of 23 days of age as compared to 104 days of age by intravenous injection. A smaller infecting dose might have allowed expression of a degree of age resistance. Also, if a wider range of birds had been used the results might have been different.

The trial summarized in table 2 indicates that under experimental conditions the size of the infecting dose by intravenous administration has no appreciable effect on the amount of CTC necessary to prevent subsequent development of the disease. A 0.10 ml dosage was necessary to produce lesions

during the holding period of 14 days. Doubling the dosage in progressive groups of birds to a maximum of 1.6 ml of yolk-propagated agent decreased the incubation period from 8 to 3 days but otherwise had no noticeable effect. A 75 gram per ton level of CTC previously determined to be only slightly over the minimum required prophylactic level necessary for this strain of the agent was effective in suppressing lesions in the paired medicated groups at all infecting levels.

The age of trial birds used to evaluate the prophylactic or therapeutic value of a given level of a drug administered in the feed for systemic effect probably has an important influence on the results. Although the age of the bird per se may influence results, the effect of feed consumption in relation to body weight at different ages probably is more significant. Field data indicates that chickens on a modern, low fiber, 4 percent added fat ration will consume approximately 1 pound of feed per pound of body weight during the first week of life. The feed intake per pound of body weight steadily decreases with increase in age and by the 10th or 12th week approximately 1 pound of feed is consumed for each 2.5 pounds of body weight. It would thus appear that approximately 2.5 times the concentration of a medicament which acts systemically would be required in the feed to secure a comparable drug concentration in the body during this period as compared to the 1st week of a chicken's life. Similarly, the concentration of a given drug in the feed to prevent lesions at 12 weeks of age would be calculated to be approximately 50 percent more than that required to prevent lesions during the 5th week as in most of these trials. The total feed intake as compared to body weight is also influenced by a variety of other factors including energy level of a ration; feed intake increases as the energy level of a ration decreases.

A considerable difference was observed in the drug sensitivity of several of the isolates tested. Under the conditions of these trials, birds were completely protected against several isolates by a 50 gram per ton CTC feed medication; birds infected with other isolates were protected at a 200 but not at a

150 gram per ton CTC feed level. Quite similar variability in sensitivity to OTC and furazolidone was noted. These relatively wide differences in sensitivity may be assumed to indicate strain differences. On this basis, the seven isolates used represented at least two strains. The relative sensitivity of all isolates to CTC, OTC and furazolidone was approximately constant. In all trials CTC was superior to OTC and furazolidone was considerably less effective than either of these tetracyclines. The possibility of other strains producing different sensitivity patterns should not be completely overlooked.

The relative value of dihydrostreptomycin by injection as compared to feed medication of tetracyclines was difficult to determine. Subcutaneous injection of dihydrostreptomycin rather early in the incubation period is quite effective, but if administered late in the incubation period or when signs are shown its effectiveness is greatly reduced. The incubation period varies depending upon the conditions of infection and so it is not possible to determine from these trials the time interval during which dihydrostreptomycin is effective following infection. Additional trials are necessary to evaluate more critically the value of dihydrostreptomycin in the control of this disease.

The agent of infectious synovitis is slightly sensitive to cathomycin but has little or no sensitivity to erythromycin.

No important difference was found between chickens and turkeys in relation to the rigns or lesions of the disease or in the behavior of the prophylactic drugs tested.

The selection of a feed medication level for use in field outbreaks must be quite arbitrary because of differences in strain sensitivity of the agent and variable feed intake in relation to body weight. Field observations indicate that a 200 gram per ton level of CTC is usually adequate to contain an infection during the period of medication. Interpretation of these trials on a basis of anticipated feed intake in relation to body weight together with variable drug sensitivity of the infecting agent suggests that under field conditions, a feed level varying from 50 to over 300 grams per ton of CTC

could be the minimal amount necessary to contain the disease. These considerations probably explain the apparent differences in sensitivity of the agent to prophylactic feed medication reported by some investigators.

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SUMMARY

1. A marked variation in drug sensitivity existed between some strains of infectious synovitis.
2. The response of turkeys to prophylactic medication for infectious synovitis was essentially the same as for chickens.
3. No difference in susceptibility to infectious synovitis of chickens between 23 and 104 days of age was found.
4. The amount of the agent used for infection modified the length of the incubation period but had no direct relationship to the amount of chlortetracycline necessary to suppress appearance of lesions.
5. Chlortetracycline was consistently more effective as a prophylactic feed medication against all isolates of infectious synovitis tested than was oxytetracycline; furazolidone was consistently less effective than was either of these tetracyclines.
6. Dihydrostreptomycin was effective if administered subcutaneously early in the incubation period.
7. Cathomycin per os possesses some activity against the agent but erythromycin either per os or subcutaneously had little or no activity.

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CASE REPORT—ROUND HEART DISEASE IN THE UNITED STATES*

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So called round heart disease in chickens has been reported from England, Ireland, Australia, New Zealand, and most European countries.[7,10] Vance[4] indicated that the disease has been present in Canada for at least six years, although it was not recognized until 1954. Since then, 30 cases have been found. Bankier[4] reported the presence of the disease in British Columbia in 1953. No report of round heart disease in the United States is known to the writer other than the cases recorded from this laboratory.[1,2,3]

The case histories of the many outbreaks described in the literature follow a remarkably similar pattern.[10] The

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disease most often has an acute or peracute course. Birds in good condition, with no signs of abnormality, die suddenly, usually after some exertion. Deaths often occur in the owner's presence while the birds crowd about during feeding time. The mortality varies widely, ranging from zero to 40 percent or more. Wilson[10] indicated that in some outbreaks, individual birds may have slight cyanosis of the combs and be depressed for several days before succumbing. Mortality usually stops spontaneously.

The cause of round heart disease has not been determined. All efforts to involve an infectious agent have failed. Genetic or nutritional factors have not been incriminated. Zinc poisoning was suspected[5] but was subsequently discounted.[6] Some healthy, normal birds introduced into an affected flock for 30 and 47 days have succumbed to the disease.[8,11] However, the fact or factors responsible for this transmission were not uncovered.

It is the purpose of this report to record the first cases of round heart in the United States and to describe efforts that were made to alter the course of one outbreak.

CASE RECORDS

Accessions 36200, 36235. The first outbreak of round heart disease was recognized in January, 1949. Forty birds out of a flock 121 nine-month-old pullets of the White Leghorn breed had died within a month. For the most part the birds were in good flesh and died suddenly. Some birds became progressively emaciated.

Four dead birds were submitted. Three were thin while the fourth was in good flesh. Two birds had a marked hydropericardium. The internal organs were congested but with the exception of the heart were otherwise normal.

The hearts were markedly enlarged and grossly misshapen to a rounded or barrel shape (figure 1). This was largely due to the marked thickening chiefly of the left ventricular wall, although the right ventricle was also affected. The heart muscle was pale in color with whitish streaks in

the muscle bands. There was an obvious venous congestion throughout. Efforts to isolate organisms by bacteriological methods from the heart and other organs failed.

Accessions 54658, 54667. A second case occurred in March, 1955. Fifty year-old White Leghorns had died out of a flock of 440 birds within one week. Production had dropped from 70 percent to 50 percent in one week. Deaths were very sudden and the birds were all in good flesh. Again the hearts presented the same appearance as in the first case. Culture attempts from the internal organs were unsuccessful.

Accessions 56037, 56058, 56090. The outbreak recorded in these accessions occurred on the same farm where the second case had been found. Losses began in May, 1956, in a flock of 350 White Leghorns and continued until the end of August. The outbreak followed the same pattern as in the previous year. Deaths occurred suddenly, most often at feeding time. The peak of losses took place in June, with 18 percent total mortality directly attributable to round heart disease.

Many severe cases of round heart were encountered. For the first time, markedly enlarged hearts with a dimple in the musculature at the apex were encountered. There were birds with large amounts of ascitic fluid in the abdominal cavity. Chronic passive congestion of the liver was found several times. Extreme engorgement of the venous system was seen in a number of birds.

EXPERIMENTAL

The affected flock was kept in a single pen with a deep built up litter of wood shavings. The litter was dry and in good condition. The management and care of that flock was considered entirely adequate. An effort was made to alter the course of the disease. The pen was divided into equal parts with a chicken wire partition. Pen A with 163 birds, the control, was fed a commercial ration and was watered as in the past. This pen was supplied from a well which yielded water with a slightly sulfurous taste. This water was used in the dwelling and had been the water supply in this chicken pen for many years in the past. Pen B was to have been

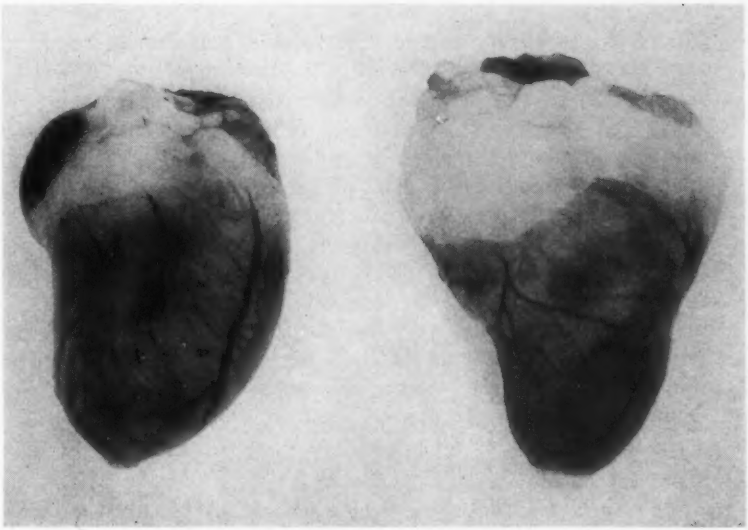

Fig. 1. (Left) Heart from chicken affected with round heart disease. (Right) Heart from normal chicken for comparison.

handled in the same way, except that a different commercial feed was to have been supplied. However, the owner saw fit also to water the birds from a different source than in A—from a creek that ran by the house (table 1).

Five days after dividing the pen, 40 birds were selected at random from each of the two pens and removed to a clean pen with clean litter near the laboratory. These 80 birds were kept on the same feed as at the home farm for 37 days. For the following 73 days, they were fed a different commercial ration, the same as fed to pen B.

Twenty yearling laboratory reared White Leghorn hens were placed in a pen with about four inches of litter removed from the affected farm. Every day a substantial amount of the litter was mixed in the feed to force the hens to consume this material. The birds were exposed to this litter for 163 days.

RESULTS

All the mortality recorded in table 1 was due to round heart disease. The total mortality in the control pen A was more than twice that of pen B, which had been receiving different feed and water. Statistically (Chi square) this difference was highly significant. Despite the fact that 51 days after the beginning of this trial all the litter from pen A, the most severely affected, was transferred to pen B, the disease subsided spontaneously in both pens.

The 80 birds, half of which were procured from each of the two pens, experienced no mortality from round heart during the 73 days they were held on the laboratory grounds. This was entirely unexpected since the heaviest mortality on the home farm occurred during this period.

Egg production throughout the course of the experiment varied from a high of 66 percent to 34 percent on the home farm. The control pen outlayed the experimental pen by an average of 5.5 percent per day. During the period June 22 to August 27, pen A averaged 7.6 percent more eggs daily than pen B. This was also unexpected since pen A suffered the greatest mortality during this same period. Grossly and histologically, the lesions in the heart matched the description given by Wilson.

The twenty laboratory reared yearling hens, kept on the litter from the affected flock for 163 days, did not succumb to round heart disease.

To check on the possible toxicity of the feed given to this flock, a number of poultry farms using the feed from the same source were visited. No unusual losses were reported from these flocks. A few sick or dead birds from these farms were examined but no evidence of round heart disease was seen.

Pieces of hemlock bark were found in the wood shavings that were used for litter on the affected farm. To check on the possible toxicity of this material, 355 grams of ground hemlock bark was fed in capsules to a caged White Leghorn hen over a 35 day period. This bird did not sicken or slacken in egg production. At necropsy, no evidence of round heart was found.

TABLE I

Course of a field outbreak of round heart disease in a divided pen, each half of which received feed and water from different sources.

Pen A—Control				Pen B—Different feed and water			
No. birds		Mortality		No. birds		Mortality	
163	June 17 to June 22 40 birds removed June 22	3	1.8%	163	June 17 to June 22 40 birds removed June 22	6	3.6%
120	June 22 to August 7	23	19.1%	117	June 22 to August 7	6	5.1%
110	August 7 to September 24 Old litter removed New litter used.	2	1.8%	111	August 7 to September 24 Received old litter from Pen A.	1	1.9%
TOTAL		28	23.0%	TOTAL		13	10.0%

DISCUSSION

We were unsuccessful in producing round heart disease in normal birds placed on litter from the affected farm. Wilson[11] reported success in such an attempt wherein six birds out of 74 came down in 42 to 95 days. The failure of any of the 80 birds from the affected flock to develop the disease after being moved to a pen with clean litter matched Wilson's experience. Wilson called attention to the fact that most of the cases he observed occurred in flocks running on deep litter and in one instance, one pen of birds on damp and "cloggy" litter was affected. Another pen of comparable birds, on the same farm, but on a dry and "well-made" litter, was normal. In our case, the deep, built up litter was dry and in excellent condition.

Despite the marked reduction in mortality (statistically significant) in pen B, it is difficult for the writer to ascribe epizootiological significance to this finding. Investigation of the mortality on farms using feed from the same source did not incriminate feed as a possible cause. Furthermore, feed from the same source, given for 37 days to 80 birds moved from the affected to a clean pen, did not result in any cases of round heart.

Eliminating the feed as a causative factor would leave

the water as a possible cause since pen B received water from a creek. Although the disease had occurred on this same farm the previous year, no difficulties had been encountered in the many years before when the same well water had been used.

SUMMARY

1. The first cases of round heart disease in the United States are reported. Three outbreaks, two on the same farm in successive years, are recorded from New York State farms.

2. Efforts were made to alter the course of one outbreak by dividing an affected flock into two pens. One pen served as a control, while the other received feed and water from a different source. The control pen suffered a mortality from round heart disease of 23 percent as against a mortality of 10 percent in the other pen. Although the difference is statistically significant, it is difficult to ascribe epizootiological significance to these results in the light of the history of the poultry operation on the affected farm.

3. Eighty birds, selected at random from the affected flock, and moved to a pen with clean litter on laboratory grounds, suffered no losses from round heart.

4. An attempt to reproduce round heart disease in normal chickens by keeping them on litter from the affected pen failed.

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ABSTRACTS OF PAPERS PRESENTED AT 30th NORTHEASTERN CONFERENCE ON AVIAN DISEASES, JUNE 30, JULY 1 AND JULY 2, 1958, CORNELL UNIVERITY, NEW YORK STATE VETERINARY COLLEGE, ITHACA, NEW YORK

A FURTHER INDICATION OF EGG TRANSMISSION OF INFECTIOUS SYNOVITIS

G. H. Snoeyenbos and H. I. Basch
University of Massachusetts, Amherst, Massachusetts

Complete manuscript printed elsewhere in this issue.

INFECTIOUS SYNOVITIS I. CLINICAL AND PATHOLOGICAL MANIFESTATIONS

Martin Sevoian, G. H. Snoeyenbos, H. I. Basch, and Iona M. Reynolds
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INFECTIOUS SYNOVITIS II. DRUG PROPHYLAXIS AND THERAPY

G. H. Snoeyenbos, H. I. Basch, and M. Sevoian
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THE BIOLOGICAL VARIATION OF THE INFECTIOUS LARYNGOTRACHEITIS VIRUS

M. S. Cover and W. J. Benton

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THE CLINICAL AND SEROLOGICAL RESPONSE OF CHICKENS TO CERTAIN LARYNGOTRACHEITIS VIRUSES

W. J. Benton, M. L. Cover, and L. M. Greene
University of Delaware, Newark, Delaware

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RESPIRATORY FLORA AND DISEASES OF ANTARCTIC BIRDS

John McNeill Sieburth
Virginia Agricultural Experiment Station, Blacksburg, Virginia

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A REEVALUATION OF THE USE OF MEDIA FOR THE ISOLATION OF PLEUROPNEUMONIA-LIKE ORGANISMS OF AVIAN ORIGIN

Julius Fabricant
Department of Pathology and Bacteriology, N. Y. State Veterinary College, Ithaca, N. Y.

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A STREPTOBACILLUS THE CAUSE OF TENDON SHEATH INFECTION TURKEYS

C. I. Boyer, Jr., D. W. Bruner, and J. A. Brown
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FURAZOLIDONE MEDICATION IN CHICKENS EXPERIMENTALLY INFECTED WITH *SALMONELLA PULLORUM*

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HATCHERY SPREAD OF PULLORUM DISEASE THROUGH DEBEAKING

R. E. Jacobs, M. N. Frazier, and M. E. Tourtellote
University of Connecticut, Storrs, Connecticut

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SALMONELLA ORGANISMS ISOLATED FROM POULTRY FEED

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THE PROPAGATION AND CYTOPATHOGENIC EFFECT OF AN EGG ADAPTED STRAIN OF INFECTIOUS BRONCHITIS IN TISSUE CULTURE

T. W. Chomiak, R. E. Luginbuhl, and E. L. Jungherr
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IN VITRO RESISTANCE OF PPLO TO ANTIBACTERIAL AGENTS

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CONTROL OF INFECTIOUS SYNOVITIS XI THE POTENTIATING EFFECT OF TEREPHTHALIC ACID ON CHLORTETRACYCLINE

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West Virginia Agricultural Experiment Station, Morgantown, West Virginia

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CASE REPORT—ROUND HEART DISEASE IN THE UNITED STATES

P. P. Levine
New York State Veterinary College, Ithaca, New York

Complete manuscript printed elsewhere in this issue.

INFECTIOUS LARYNGOTRACHEITIS STUDIES

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Six groups of 2, 3, 5, 7, 9 and 11-week-old chickens were used. Fifteen chicks of each group were infected intratracheally with 5 vaccine doses of the virus. Five chicks were left as susceptible, contact controls. Respiratory symptoms appeared in the inoculated birds 2 days after exposure and mortality started by the 4th day. In the susceptible contact controls, symptoms were delayed and appeared 8 days after ex-

perimental birds were inoculated. Eye and sinus involvement was seen early and the virus was isolated from the sinus and eye discharge. Ten, 9, 8, 8, 7 and 13 chickens died from the 2, 3, 5, 7, 9 and 11-week-old infected groups, respectively. Of the 5 contact control birds per group, 2 died in each of the 5, 7, 9 and 11-week-old groups. The 2 and 3-week-old control groups suffered no mortality.

All the birds in the different age groups were susceptible to the infection. The symptoms in the 2 to 3-week-old groups were mild and no blood casts were seen in the tracheas. The virus was isolated from the dead birds in each group.

Two and 3 weeks after exposure, the survivors from the infected groups were mixed with an equal number of susceptible birds of a similar age to determine if transmission would take place. Some of the infected chickens were still showing respiratory rasping at the time they were mixed with the susceptible birds. No respiratory symptoms were observed during the 8 weeks of observation.

Six groups of 2, 3, 5, 7, 9 and 11-week-old chickens were vaccinated with commercial laryngotracheitis vaccine. The vaccine was administered cloacally. After 3 weeks each individual chicken received 5 vaccine doses of the virus intratracheally. All the birds, twenty per group, withstood the challenge.

Contribution 952 from the Animal Pathology Department, Agricultural Experiment Station, University of Rhode Island.

HATCHERY SANITATION

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Some specific and non-specific ailments in chicks and poults commencing at 10 days to 2 weeks of age indicated the need for a sanitary survey of the hatcheries involved. In some cases, the ailments existed several months before the sanitary survey was requested. Mortality was between 3 and 10 percent and treatment had been varied with little amelioration. Microbiological examination of fluff, dust and air throughout the hatchery frequently revealed several millions of microorganisms. Sometimes almost pure populations of *Aspergillus spp.* were found. Otherwise mixed populations with a high percentage of *Staphylococcus spp.* occurred. The examination of fluff samples from the hatcher was the best index of sanitation of the hatchery and indicated the microbial load to which birds were exposed during confinement in the hatcher.

Alterations in sanitation in some hatcheries drastically reduced early mortality. Usually better sanitary practices and more effective disinfection were both necessary since too much dependence had been placed on disinfectants and deodorants. From this work a plan has been evolved to assist hatcheries in the maintenance of a uniformly high level of sanitation throughout Ontario. Fluff samples are being collected regularly at quarterly intervals by hatchery inspectors for laboratory analysis. Recommendations are made after consideration of the laboratory findings and the inspectors' reports. It is anticipated that this plan will prevent certain specific and non-specific ailments of birds which in the past have been associated with high microbial populations which developed obscurely in hatchers under divers conditions.

THE INCIDENCE OF NEURAL LEUCOSIS AS AFFECTED BY MANAGEMENT

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White Rock chicks from the same group of parents were brooded

on the floor in 6 separate pens. Group 1 was gravel floored and held roosters until one month before disinfection with lysol and brooding. Group 2 was gravel floored and held roosters until one month before disinfection with Wescodyne and brooding. Group 3 was cement floored and held roosters until six months before disinfection with lysol and brooding. All birds which became sick or died were necropsied. Diagnosis of leucosis were made on the basis of gross lesions. Mortality due to neural leucosis for 365 days was 7.2 percent group 1; 18.5 percent group 2 and 5.7 percent for group 3.

The next year (1958) the entire brooding area was fumigated with formaldehyde, left vacant for three months and disinfected with cresol. The one caretaker was allowed only in the brooding area where he wore overalls and boots. The boots were disinfected on entering and leaving with saponified cresol. Three groups of White Rocks and one group of commercial hybrids were kept for 9 weeks. All losses due to leucosis were recorded on the basis of gross lesions. Of 2466 White Rock birds raised on concrete floors none developed leucosis. Of 2124 White Rock birds raised on dirt floors, about 4 percent developed leucosis. Of the 2100 hybrids, raised on concrete floors none developed neural leucosis by the 5th week.

These results indicate that the virus of leucosis can remain viable on the floor of brooder houses for at least 6 months. Older birds should not be housed in rooms used for brooding. Disinfection with cresol reduces the incidence and it is possible to almost eliminate infection.

PATHOLOGICAL ASPECTS OF POULTRY INSPECTION UNDER THE POULTRY PRODUCTS INSPECTION ACT

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Since August 28, 1957, when Congress took final action for passage of the Poultry Products Inspection Act, there has been increased activity on the part of the Inspection Branch, Poultry Division, Agricultural Marketing Service, as it has the responsibility of carrying out the provisions of the laws.

Industry is showing a cooperative spirit in preparation of facilities and the necessary reorganization for operating in compliance with requirements of the Act.

Sections 2 and 3, in part, read as follows: "Wholesome poultry products are an important source of the Nation's total supply of food. Unwholesome and adulterated poultry products in the channels of interstate or foreign commerce, are injurious to the public welfare, adversely affect the marketing of wholesome poultry products, result in sundry losses to producers, and destroy markets for wholesome products."

Section 3. "It is hereby declared to be the policy of Congress to provide for the inspection of poultry and poultry products by the inspection service as herein provided to prevent the movement in interstate or foreign commerce or in a designated major consuming area of poultry products which are unwholesome, adulterated, or otherwise unfit for human food."

The Inspection Branch has no formal responsibility for disease control and eradication, treatment or research except that which is provided for under quarantine sections of the Act, and the Regulations. Inspectors of the Inspection Branch, Poultry Division, condemn poultry carcasses and parts based on indications of the nature, extent and duration of disease processes. These indications, such as cyanosis or

anemia of the musculature, dehydration, loss of normal tissue tone and sheen, are considered as evidences of unbalanced metabolism and deviations from normal physiological functions. In general inspectors do not make specific diagnoses excepting in well characterized conditions like tuberculosis or leukosis for example. Most condemnations are recorded under such headings as septicemia-toxemia, inflammatory processes or some other general type of condition indicative of unwholesomeness.

When inspectors, by reason of ante mortem and/or postmortem inspection evidences, suspect presence of a disease transmissible to man, they seek the cooperation of appropriate local and Federal agencies as directed by official instructions under authority given in Section 6 of the Act.

The responsibility for wholesomeness of poultry rests, not only with the Inspection Branch, but also with the poultry industry in all its phases.

THE SEROLOGICAL RESPONSE AND IMMUNINTY TO EXPERIMENTAL NEWCASTLE DISEASE VACCINE

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Over a two year period, 234 commercial Newcastle (ND) vaccines collected from poultry supply outlets througout Maine by state officials have been examined for hemagglutination (HA) activity and embryo infectivity in relation to storage conditions. This study revealed that some vaccines were outdated and not viable.

The HA varied greatly in vaccines of certain types and also with those having the same company serial number. Studies showed that incubation for varying lengths of time at 37°C was very detrimental to the HA activity.

Of 32 establishments selling ND vaccine, 25 used commercial refrigerators for storage, one stored vaccine with commercial groceries (refrigeration), one each stored it on grain bags, in a coke cooler, in a deep freezer, in a refrigerator with dry ice, in a cool cellar and one lot had been stored three weeks at room temperature.

Experiments were conducted with B_1, Roakin and LaSota strains of ND by passing the virus in embryos and immediately after harvest, titering the aa fluid. Vaccines were prepared at 1:640, 1:320, 1:160, 1:80 and 1:40 HA titers. The chickens were given a one drop intranasal vaccination and bled daily for at least 30 days. Hemagglutination-inhibition (HI by alpha technique) and serum neutralization tests were conducted on all sera. The birds were challenged with a pathogenic strain of ND, at the end of the test period. All the vaccines down to and including the 1:40 HA titer vaccine gave serological immunity. A field trial on 9000 birds showed that spray vaccination with a 1:40 HA vaccine gave good serological response and immunity to challenge.

CASE REPORT. CLINICAL EVIDENCE SUGGESTING THE POSSIBILITY OF EGG TRANSMISSION OF AVIAN HEPATITIS

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New York State Veterinary College. Regional Veterinary Laboratory, East Aurora, New York and New York State Veterinary College, Ithaca, New York

Two groups of chicks five days and fourteeen days of age hatched in two widely separated hatcheries from eggs produced by a single hepatitis infected supply flock were submitted to the East Aurora laboratory with symptoms of avian encephalomyelitis. Post-mortem examinations revealed liver lesions indistinguishable macroscopically and microscopically from those of avian infectious hepatitis. Bacteriologicial examinations of these livers were negative.

In some trap-nested birds of another breeder, poor hatchability was reported in the presence of good fertility. Examination of embryos revealed liver lesions indistinguishable from those seen in this disease.

In a third flock where the hatchability was poor, the livers of pipped embryos and embryos which died towards the end of the incubation period had similar lesions. Subsequent examination of some of the breeders revealed macroscopic and microscopic lesions attributed to avian infectious hepatitis.

It is recognized that since attempts at isolation of the causative agent were not done, proof of egg transmission of infectious hepatitis cannot be claimed. However, circumstantial evidence presented here suggest the strong possibility that such transmission may occur.

INCIDENCE OF CASEOUS EXUDATE IN THE AIR SACS OF CULL CHICKS AND THE EMBRYOS OF PIPPED EGGS FOLLOWING ANTIBIOTIC INJECTION OF THE BREEDERS

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Cull chicks and embryos from pipped eggs were examined from 39 hatches in one hatchery during the period of December 16, 1957 to May 5, 1958. A total of 562,195 eggs were set of which 74.5 percent hatched. From these 2,379 cull chicks and 3,962 embryos from pipped eggs were examined. Caseous exudate was found in the air sacs of 6.6 percent of the culls and 8.6 percent of the embryos. The difference, embryos versus culls, approaches statistical significance. The ratio of lesions to no lesions was the same for live and dead culls and for live and dead embryos.

A total of 212 cultures were taken; 143 showed contamination and 29 revealed PPLO. Only 3 PPLO isolations were obtained from embryos where no bacteria were isolated. PPLO isolations were recovered more frequently from the dead culls and embryos than from the live.

Injections of the dams with Duo-Strep, 200 mg., Spiramycin 100 mg., or 75 mg streptomycin and 15,000 units of penicillin did not reduce the incidence of air sac lesions in culls or embryos.

THE PATHOLOGY OF CHICKEN EMBRYO INOCULATED WITH CERTAIN AVIAN VIRUSES

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The viruses of avian encephalomyelitis (AE), synovitis, Newcastle disease (ND), infectious bronchitis (IB), pleuropneumonia-like organism of chronic respiratory disease and infectious turkey sinusitis, and the agent of avian infectious hepatitis (AIH) were inoculated into chicken embryos for the purpose of determining the pathologic response of this host system. Killed embryos were examined at 24 hour intervals for an average of 10 days postinoculation and subjected to histopathologic examination at 8 cross section and 1 parasagittal level. A total of 1000 infected embryos were employed with 400 diluent-inoculated embryos serving as controls. AE caused dwarfing of the embryos and microscopically, neuronal degeneration in the ventral horns of the spinal cord and dorsal ganglia. Skeletal muscles showed extensive dystrophy and the CNS edema, encephalomalacia and terminal internal hydrocephalus. Synovitis produced splenomegally and hepatomegally, marked perivascular reticular cell proliferation in the liver and occasionally in the gizzard, skeletal muscles and mesonephros. PPLO, consisting of hemagglutinating and non-hemagglutinating strains, produced heterophilic periarteritis and periphlebitis in the liver and terminally in the

lung. Livers of AIH infected embryos had a greenish cast and microscopically, showed early focal necrosis, occasional aggregates of heterophils accompanied by bile duct proliferation, bile stasis and regenerating liver cord cells about the hepatic vessels. ND and IB viruses to date have not produced consistent microscopic lesions in the embryo possibly because of the employment of embryos from immune dams. The pathologic response of the chicken embryo is considered contributory to the differentiation of certain avian viruses.

REPORT OF THE COMMITTEE ON NOMENCLATURE AND REPORTING OF DISEASES NORTHEASTERN CONFERENCE ON AVIAN DISEASES JUNE 1958

The purpose of this report is to present information on the incidence of disease in the area encompassed by this Conference. Furthermore it is hoped that a nomenclature and system of reporting of diseases can be developed which may serve to standardize the reports from the various laboratories.

The statistics are based on the calendar year 1957 with the exception of those for Canada and Virginia which are based on a fiscal year. Each unit statistic represents a single case or consignment to the laboratory and therefore approximates an outbreak of disease on a premises. The figures are reported by states rather than by member laboratory. Statistics shown for Canada were compiled from those submitted by the Ontario Veterinary College, Guelph and the Regional Laboratories at Ridgetown and Kemptville.

The incidence of disease is reported for age groups which coincide roughly with the brooding, growing and adult life of the birds. A separate category indicates incidence of disease in species other than chickens. Whenever possible the identity of the species is indicated: T= turkey, Ph = pheasant, D = duck, Pkt = parakeet, Gse = goose, Gu = guinea, Pgn = pigeon, WD = wild duck, Par = parrot, RG = ruffed grouse and GK = grackle. More than one species was combined and designated by letter O.

All infectious and contagious, parasitic and nutritional diseases plus such miscellaneous and unclassified conditions and diseases as may be of significance or interest are reported. However limitations of space and variations in systems of reporting by the various laboratories make it necessary to be somewhat arbitrary in preparing the report and some discrepancies and omissions are unavoidable.

Specific nomenclature is used wherever possible to indicate specific disease entities. However, for expediency some classifications are used in a generic sense and singleness or identity of etiology is not indicated. Ascaridiasis, coccidiosis and pox are examples. Nutritional diseases are classified according to clinical syndrome. Thus under the term rickets are included cases of improper calcification of bones whether due to avitaminosis D, a deficiency or imbalance of minerals as calcium or phosphorus, or an excess of sulfur in the diet. The statistics are presumed to be essentially accurate even though not all diagnoses are based upon the isolation or identification of the etiological agent.

Certain unavoidable discrepancies are indicated by letter which also appears by the statistic to which it applies.

a—figure includes all chickens regardless of age
b—type of roundworm not specified
c—figure includes all types of Leukosis

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Summary of Poultry Diseases
Reported by Northeastern Conference of Laboratory Workers

State		Conn.	Del.	Maine	Mass.	N.H.	N.Y.	N.C.	Penn.	R.I.	Vt.	Va.	W.Va.	Canada
Consignment Totals		1321	1986	1162	1650	2907	6101	2665	5192	209	377	4384	1500	4153
Ascaridiasis	1-4 Wks	a 27					1		a 393		a 5	a 77		3
	1-5 Mos		b 32	21	1	2	70	148					78	109
	+ 5 Mos		b 11	52	13	25	275	18		2			10	291
	Other		T-5	c.G.4	0-2	T-1	I-2	0-27	T-4			T-22		T-4
Aspergillosis	0-4 Wks		24	1	1		13	2	a 22		a 1	a 19	16	7
	1-5 Mos		5		1		26	13					1	6
	+ 5 Mos				3		16						4	4
	Other		T-9	T-2	0-5		0-23	T-17	0-30			0-23	T-1	0-49
Avitaminosis A	1-5 Mos						8		a 3			a 3		1
	+ 5 Mos				1		23			1				1
	Other				T-1									T-4
Blue Comb Complex	0-4 Wks	a 16	2					1	a 130		a 5	a192	2	
	1-5 Mos		18		1	1		11					22	9
	+ 5 Mos		10		17	12	41	6		1			15	43
	Other		T-5		T-7	T-1	T-16		T-21			T-668	T-46	T-1
Bronchitis, Infectious	0-4 Wks	a 38	11		2	5	18	4	a 129	3	a 4	a 54		21
	1-5 Mos		105	38	19	77	55	16		13			10	15
	+ 5 Mos		8	7	12	53	52	19					2	14
Capillariasis	0-4 Wks	a 6												1
	1-5 Mos			10	8	10	19	66	a 224		a 1	a 4		49
	+ 5 Mos			67	39	81	187			1			13	128
	Other						0-5	T-1	0-14	Pgn-1			Pgn-2	0-3
Cholera, Fowl	0-4 Wks	a 2	1						a 110		a 2	a 15		1
	1-5 Mos		8		1		2	1					8	3
	+ 5 Mos		15	7	17	9	30	14						19
	Other		0-10		0-6	T-1	0-75	0-6	0-16	Ph-2	T-1	T-77	T-29	T-11
Coccidiosis	0-4 Wks	a 69	1	2	5	1	17	17	a 386		a 17	a 255	4	30
	1-5 Mos		85	72	78	87	398	121		14			93	236
	+ 5 Mos		4	16	18	47	171	58		1			1	86
	Other	0-8	T-20		0-17	T-2	0-39	0-133	0-59	0-3	0-2	0-205	T-64	0-75
Coryza, Infectious	0-4 Wks		2						a 11		a 5	a 7		
	1-5 Mos		5			1		7					3	3
	+ 5 Mos		1		1	2				1			2	9

Code: a - figure includes all chickens regardless of age
b - type of roundworm not specified

b - type of roundworm not specified

State		Conn.	Del.	Maine	Mass.	N.H.	N.Y.	N.C.	Penn.	R.I.	Vt.	Va.	W.Va.	Canada
Consignment Totals		1821	1986	1162	1650	2907	6101	2665	5192	209	377	4384	1500	4158
Encephalomalacia	0-4 Wks	a 9	1					1	a 7		a 1		1	
	1-5 Mos						1	6					1	
	+ 5 Mos							2						
	Other	T-1					T-1		T-1		T-1			
Encephalomyelitis, Avian	0-4 Wks	a 47	52	16	27	27	72	5	a 96	2	a 9	a 44	7	44
	1-5 Mos		5		3	3	9	62					13	14
	+ 5 Mos													1
Enteritis, non specific	0-4 Wks	a 3	9	1					a 73		a 1	a 163	3	39
	1-5 Mos		94	24	2	20	44	58		1			1	107
	+ 5 Mos		24	18	5	33	4	6		6			1	105
	Other		0-42	0-3		T-1	0-31	T-69	0-63	T-1		0-293		0-95
Enteritis, ulcerative	1-5 Mos	a 7			11		20							
	Other				T-2		0-7	0-3	Q1-2	Q1-1				0-8
Erysipelas	1-5 Mos										a 1			2
	+ 5 Mos						4							1
	Other	T-2	T-3		T-24	T-1	T-13	T-57	T-26		T-1	T-90	T-41	T-44
Hemorrhagic syndrome	0-4 Wks	a 12					2	1	a 132			a 62		64
	1-5 Mos		74	4	22	9	173	10					1	163
	+ 5 Mos		1	2	5	5	10			3				14
	Other	T-2					0-3		T-1			T-34		T-18
Hepatitis, Duck Virus							D-153							D-1
Hepatitis, Infectious Avian	1-5 Mos	a 140		6	17	5	12		a 23		a 2		1	3
	+ 5 Mos			12	59	3	154			1				2
Hexamitiasis	0-4 Wks												1	
	1-5 Mos												2	
	Other		T-2		T-1				T-2			T-3	T-46	
Histomoniasis	0-4 Wks	a 9	1				4	4	a 117		a 4	a 17		8
	1-5 Mos		20		17	5	187	57					4	95
	+ 5 Mos		3		5		34	19						26
	Other	0-7	T-56		0-31	T-7	0-46	Ph-1	0-70	T-1		T-169	T-50	0-79
Laryngotracheitis, Infectious	0-4 Wks	a 103	3						a 15		a 2	a 52		2
	1-5 Mos		114	5	4	2	1			4			12	13
	+ 5 Mos		7	14	13	8	8			6				10
Leucocytozoon infection				WD-1							D-1	T-6	T-3	

Code: a - figure includes all chickens regardless of age.

State		Conn.	Del.	Maine	Mass.	N.H.	N.Y.	N.C.	Penn.	R.I.	Vt.	Va.	W.Va.	Canada
Consignment Totals		1821	1986	1162	1650	2907	6101	2665	5192	209	377	4384	1500	4158
Osteomalacia	+ 5 Mos				5		12		a 5					
	Other	T-1					Pgn-1							
Paracolon infection	0-4 Wks		1											2
	1-5 Mos													2
	+ 5 Mos						2	5						3
	Other				T-2	T-1	D-1							0-2
Paratyphoid infection	0-4 Wks	a 18	2			2	6	13	a 4			a 14	1	10
	1-5 Mos		2	1	1		2	6					1	2
	+ 5 Mos		1		5	1	13	34						8
	Other	0-7			0-11		0-25	0-17	0-6			0-9	T-1	0-13
Pediculosis	0-4 Wks	a 1						2	a 64		a 1	a 17		
	1-5 Mos				2		3	3						
	+ 5 Mos		2	1	36	6	92	41		1			3	26
	Other				0-3		0-5		T-2					
Peritonitis	0-4 Wks	a 7					7		a 40			a 11		
	1-5 Mos		4	1	1	8	10							5
	+ 5 Mos		22	21	33	4	186	8						77
	Other				T-1		0-63	T-3				T-5		T-1
Perosis	0-4 Wks		3	1	2	2	1		a 16		a 4		2	
	1-5 Mos		3	2	1	8	27							4
	+ 5 Mos					2	1							1
	Other	T-3	T-2				0-4		T-?			T-4		0-3
Pox	0-4 Wks	a 6	2						a 77			a 21		
	1-5 Mos		4				11	11						2
	+ 5 Mos		5	1	4	6	53	26		2				7
	Other						0-4	T-2	T-6			T-10		T-4
PPLO infection	0-4 Wks	a303	44	16	4	110	27	3	a483	2	a 15	a 255	147	68
	1-5 Mos		652	181	168	188	423	152		23			158	229
	+ 5 Mos		16	86	86	93	178	29		8			25	132
	Other	T-3	T-41	0-2	T-17		0-33	T-44	0-28	T-1		T-122	T-26	0-88
Psittacosis					Par-1				0-25					0-1
Pullorum Disease	0-4 Wks	a 15	8		2		4	8	a 37		a 7	a 29	17	17
	1-5 Mos			1			3	4						6
	+ 5 Mos		2	2	6	1	13	39		1			10	11
	Other	T-1					T-3		GU-1			T-1		T-3

Code: a - figure includes all chickens regardless of age

State		Conn.	Del.	Maine	Mass.	N.H.	N.Y.	N.C.	Penn.	R.I.	Vt.	Va.	W.Va.	Canada
Consignment Totals		1821	1986	1162	1650	2907	6101	2665	5192	209	377	4384	1500	4153
Riboflavin deficiency	0-4 Wks	a 3			1		6		a 16			a 8		
	1-5 Mos		1				3	7		1				
	Other							T-1						
Rickets	0-4 Wks	a 1		4	1		4	6	a 29	2		a 19		
	1-5 Mos			6	8		5	19						1
	+ 5 Mos						4	14						
	Other		T-2			T-1	O-10		T-3	T-1		T-2		T-6
Serositis, Infectious							D-339		D-1					
Syngamus trachea					O-2		Ph-5		Ph-5	Ph-1				
Synovitis, Infectious	0-4 Wks	a 62	1	2		2	2		a129		a 3	a144		
	1-5 Mos		145	70	46	82	131	14		3			19	32
	+ 5 Mos		7	12	24	48	39	7		1				4
	Other	T-2	O-21	T-1	T-2	T-1	O-8	T-2	T-6		T-1	T-98	T-23	T-7
Tendon, ruptured gastrocremius	1-5 Mos	a 4		9	11	9	13		a 8				2	1
	+ 5 Mos			2	14	17	14							7
	Other						T-1							
Taeniasis	0-4 Wks	a 2						2	a102		a 2	a 15		
	1-5 Mos		4	2	3	3	4	3					4	11
	+ 5 Mos		10	3	12	14	34	136		5				34
	Other			WD-2	D-1		GK-1	T-19	T-1			T-1		
Trichomoniasis	0-4 Wks						1	1	a 21					
	1-5 Mos		1					2						
	+ 5 Mos		1					1						
	Other		T-3		Pgn-1				O-7					T-5
Tuberculosis, Avian	1-5 Mos						1		a 9			a 8		
	+ 5 Mos						4							32
	Other				D-1		O-2		Gse-1				Pgn-1	O-2
Tumors	0-4 Wks	a 1			1	1		2	a 30					
	1-5 Mos			10	4	7	19			1				3
	+ 5 Mos			21	5	12	133	3		3			8	3
	Other	O-8		Pkt-1	O-4		O-3		O-6		O-2			T-1
Typhoid, Fowl	0-4 Wks								a 31			a 45	1	
	1-5 Mos		3					1						2
	+ 5 Mos		4		1		2	45						10
	Other		T-1					T-5	T-3			T-21		O-2

Code: a - figure includes all chickens regardless of age

State			Conn.	Del.	Maine	Mass.	N.H.	N.Y.	N.C.	Penn.	R.I.	Vt.	Va.	W.Va.	Canada
Consignment Totals			1821	1986	1162	1650	2907	6101	2665	5192	209	377	4384	1500	4158
L	Leukemia	0-4 Wks	a 6	2				1		a 10			ac 359		
E		1-5 Mos		28			c 99	2						7	
U		+ 5 Mos		7			c209	12	1		2				2
K		Other		T-4									T-2		
O	Neural	0-4 Wks	a 82	1		1		1		a 189		a 4		2	
S		1-5 Mos		175	31	66		206	61		8			5	59
I		+ 5 Mos		6	12	19		125			23			9	40
S		Other		T-1						T-2					T-1
	Ocular	1-5 Mos	a 3	8				5		a 36					8
C		+ 5 Mos		15	2	3		54	20					6	19
C	Osteopetrosis	1-5 Mos		2	2			5		a 8		a 1			2
M		+ 5 Mos				4		15	1					1	4
P	Visceral	0-4 Wks	a 90			1		1		a 444		a 36		2	
L		1-5 Mos		139	11	12		58						37	42
E		+ 5 Mos		43	93	124		499	252		71			50	372
X		Other		T-2		T-5		T-2		T-13					0-15
Limberneck		1-5 Mos	a 3	3		1		20	3	a 11		a 2	a 5	1	
		+ 5 Mos		1	1	1	1		11						2
		Other		0-2		D-1			0-16	0-5					
Liver, Cirrhosis		0-4 Wks						1							
		1-5 Mos						1							
		+ 5 Mos				1		3							
		Other						D-58							
Mites		0-4 Wks	a 1					2		a 19					
		1-5 Mos				1		3							
		+ 5 Mos			5	13	2	42	2						
		Other				T-1		0-3							0-1
Newcastle Disease		0-4 Wks	a 116	17	1	3	1	48		a 146	2	a 1	a 29		
		1-5 Mos		184	17	9	1	71	4		8			4	
		+ 5 Mos		4	4	5	3	49	4		1			2	9
		Other	T-1	T-5		T-3			T-3	T-4	T-1		T-3		
Omphalitis		0-4 Wks	a 17	46	54	22	75	140	16	a 44	4	a 6	a 8	4	8
		1-5 Mos		3				4							
		+ 5 Mos							3						
		Other	T-1	T-1	T-1	0-5	T-2	0-40	T-6	0-13	T-3		T-5		T-4
Ornithosis								Pgn-1							

Code: a - figure includes all chickens regardless of age
c - figure includes all types of Leukosis

Avian Diseases

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Control	0	3.26	2.15	1
BACIFERM (zinc bacitracin)	4	3.34	2.16	0
BACIFERM (zinc bacitracin)	6	3.38	2.11	1
Penicillin	4	3.37	2.11	2

3 ALABAMA POLYTECHNIC INSTITUTE TEST (No. 2)

Treatment (40 birds per treatment)	Antibiotic Per Ton of Feed (grams)	Av. Wt. 7 Wks. (lbs.)	Lbs. Feed per Lb. Gain
BACIFERM (zinc bacitracin)	4	2.60	2.00
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BACIFERM (zinc bacitracin)	4	3.49	2.40
Penicillin	4	3.40	2.40

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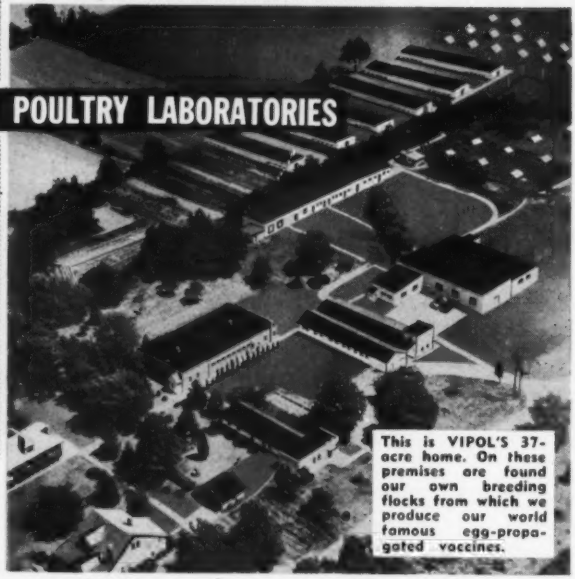

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